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Investigations on the Biologies and Immature Stages of the Cleptoparasitic Bee Genera *Radoszkowskiana* and *Coelioxys* and Their *Megachile* Hosts (Hymenoptera: Apoidea: Megachilidae: Megachilini)

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CONTENTS

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ABSTRACT

We describe for the first time the biology and immature stages (egg, mature larva, and pupa) of the cleptoparasitic bee *Radoszkowskiana rufiventris* (Spinola) based on fieldwork in Egypt over a 2-year period. The biological information includes egg deposition and hatching, developmental rates, method of killing the host immature, larval feeding, larval defecation, cocoon spinning, and voltinism. We also describe the immature stages and some aspects of the biology of the following: (1) *Megachile* (*Pseudomegachile*) *nigripes* (Spinola), the host of *R. rufiventris*; (2) *Coelioxys* (*Liothyrapis*) *decipiens* Spinola, another cleptoparasite of *M. nigripes*; and (3) *C.* (*Allocoelioxys*) *coturnix* Pérez, which attacks the nests of *M.* (*Eutricharaea*) *minutissima* Radoszkowski from the same geographic region.

On the basis of the information gained from this study and from a review of pertinent literature, we consider whether *Radoszkowskiana* and *Coelioxys* shared a common cleptoparasitic ancestor or whether cleptoparasitism evolved independently in each.

INTRODUCTION

A recent paper concluded that cleptoparasitism probably arose seven times in the leafcutter bee family Megachilidae (Rozen, 2000b). Coincidentally, Michener (2000: table 8-2) suggested that two other genera, *Radoszkowskiana* and *Bekilia*, might represent separate origins of cleptoparasitism in the family. The primary purpose of this paper is to report on the biology and immature stages of *Radoszkowskiana rufiventris* (Spinola) and to evaluate whether *Radoszkowskiana* and the other cleptoparasitic genus in the tribe Megachilini (i.e., *Coelioxys*) shared common parasitic ancestors or whether cleptoparasitism evolved independently in each lineage. The taxonomic placement of *Bekilia* remains in doubt because the type material is lost, but it is probably in the Anthidiini or Osmiini (Michener, 2000).

The tribe Megachilini contains only three genera, one of which, *Megachile*, is huge, worldwide, and composed of solitary species (Michener, 2000). *Coelioxys* is large, taxonomically complex, and wide-ranging, with its species cleptoparasitic on *Megachile*, *Trachusa*, and *Hoplitis* (representing three tribes in the Megachilinae), and on *Xylocopa*, *Anthophora*, *Centris*, *Eucera*, *Tetraloniella,* and *Euglossa* (representing two subfamilies and five tribes in the Apidae) (Michener, 2000). In contrast, *Radoszkowskiana*, consisting of only four known species, is restricted to North Africa east to Central Asia and Pakistan; its only reported host is *Megachile* (*Pseudomegachile*) *nigripes* (Spinola) (Schwarz, 2001).

This paper describes the anatomy of the eggs/mature oocytes, mature larvae, and pupae not only of *Radoszkowskiana rufiventris* but also those of its host *Megachile nigripes*

and of *Coelioxys* (*Liothyrapis*) *decipiens* Spinola. The latter species is a competitor of *R. rufiventris* in that it was also a cleptoparasite of *M. nigripes* at our study sites. Data regarding *Coelioxys* (*Allocoelioxys*) *coturnix* Pérez are added, in part because this species, though not involved with *M. nigripes*, was encountered at the nesting sites, where it attacked the nests of one or more small species of *Megachile* that used the vacated cells of *M. nigripes* as nesting sites. We thought it desirable to present whatever data are available regarding this parasitic genus for comparative consideration. Thus, immature stages and biological information of all three genera currently assigned to the Megachilini are treated.

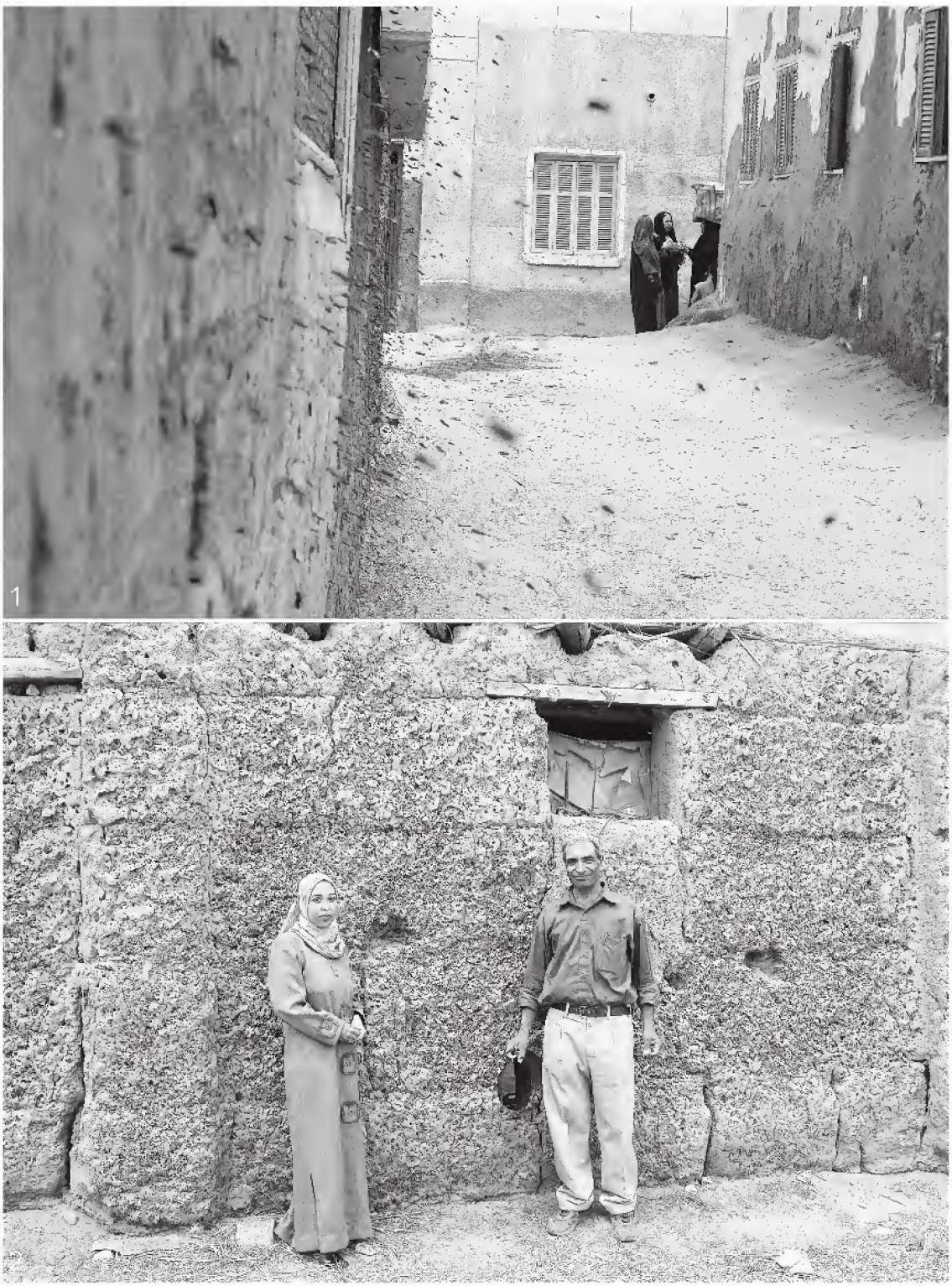
During this field investigation, we obtained immature larvae (and their cast exoskeletons) of *Coelioxys decipiens* that shed light on its mode of parasitism. Because of the complexity of this information, it is being reported separately (Rozen and Kamel, 2006), but the mature oocyte/egg, mature larva, pupa, and cocoon of *C. decipiens* as well as of *C. coturnix* are described here so that they can be compared with those of *Radoszkowskiana rufiventris* and *Megachile nigripes*. As a consequence, aspects of the biology of *C. decipiens* appear both here and in the other paper.

MATERIALS, METHODS, AND TERMINOLOGY

Fieldwork leading to this paper occurred during a 10-day period starting May 17, 2004, and during a 14-day period starting on May 20, 2005, in the vicinity of Tel el Kebir (30°33′30″N, 31°56′13″E) on the delta of the Nile River, west of the city of Ismailia, Egypt. The sites were also visited by S.M.K. alone several times during off-season to sample overwintering populations and on April 1–7, 2005, during the spring pupation period. Nest sites of the host bee, *Megachile nigripes*, were in vertical adobe walls of old houses and other old structures in a number of surrounding villages (figs. 1, 2); most of our observations were carried out in Elgezeira Elkdraa and Elkhfag. At the time of our joint fieldwork in both years, *Trifolium alexandrinum* L. (Fabaceae), the floral host of *M. nigripes*, was in maximum bloom and accounted for the great abundance of active nests of this bee in these walls. In addition to *Megachile nigripes* and its two cleptoparasites, the following species (among others) nested there and presumably collected provisions from *T. alexandrinum*: *Megachile* (*Eutricharaea*) *minutissima* Radoszkowski, *M.* (*E.*) *submucida* Alfken, *M.* (*E.*) sp., *M.* (*Pseudomegachile*) *flavipes* Spinola, and *M.* (*P.*) *cinnamomea* Alfken. Adults of *Coelioxys coturnix*, a bivoltine (or possibly multivoltine) species, attacked nests of *M. minutissima* and perhaps other small-bodied species that line their cells with leaf snippets. *Megachile minutissima* is also bivoltine (or multivoltine) and at various times of the year presumably gathers provisions from not only *T. alexandrinum* but also from *Medicago sativa* L. (Fabaceae) and an unknown yellow-flowered weed. Most immature specimens of *C. coturnix* described here, however, were taken from trap nests deployed at Suez Canal University, in Ismailia. Although these species appeared in all nesting sites in 10 or more villages, the species representation varied from site to site. In selecting sites for our excavations, we chose those where *M. nigripes* dominated to avoid confounding data relating to *M. nigripes* with those from other species. Interestingly, residents of the area, not realizing the importance of these species as pollinators of this major forage crop, consider them pests because of the damage to the "mud"[3] walls (see fig. 2). Females of *Radoszkowskiana rufiventris* and *Coelioxys decipiens* were moderately abundant, flying around the walls. Despite the relative abundance of females of *R. rufiventris*, far fewer males were collected during our studies, presumably because the species tends to be protandrous.

Immatures of host and cleptoparasites were collected two ways. To make observations on immature behavior and to collect eggs and feeding larvae, we chipped away at the adobe walls with knives and chisels and slowly made our observations and collected the immatures.

[3]The term "mud" as used in this paper refers either to construction material made of soil and liquid (water and/or perhaps nectar) in the form of (1) adobe walls constructed by humans or (2) linings of bee cells. In either case such construction material dries into a very hard substance.



Figs. 1, 2. Nesting sites of *Megachile nigripes* and it cleptoparasites in Tel El Kebir, Egypt. **1.** Abundant adults of this solitary bee and its cleptoparasites (as well as of other bees) nesting in adobe wall to the left. **2.** Kariman Mahmoud (left) and Soliman M. Kamel standing in front of adobe building showing damage caused by numerous generations of *Megachile nigripes* and other solitary bees nesting in the walls; large holes left of each of person resulted from our excavations of the nests.

We also excavated blocks (roughly 38 × 38 × 10 cm) of the adobe and transported them to the laboratory where we could more conveniently continue the slow process of chipping. To collect mature larvae and pupae, S.M.K. later found that it saved time to flow water over the block after placing it on a large screen (2 mm mesh), thus allowing sand and clay particles to wash away. Once thoroughly soaked, the block became partly disassembled so that he could easily separate cocoons from the more solid remnants of the block. He subsequently washed the cocoons in water and transferred them to ethanol.

In the description of mature oocytes and eggs, we refer to the "egg index" and to a classification of eggs based on that index. The egg index, developed by Iwata and Sakagami (1966), is calculated by dividing the length of the egg/oocyte (*E*, for egg) by the distance between the outer rims of the female tegulae (*M*, for metasomal width); that is, (*E*/*M*) (for details, see Rozen, 2003). The classification, also devised by Iwata and Sakagami (1966), is as follows:

Dwarf ($E/M \leq 0.50$);

small ($0.50 < E/M = 0.75$);

medium ($0.75 < E/M \leq 1.00$);

large ($1.00 < E/M \leq 1.10$);

giant ($1.10 < E/M$).

In attempts to examine eggs of the treated species by recovering mature oocytes, we dissected fresh females after they were killed by rupturing the intersegmental membrane between the second and third or third and fourth metasomal segments with forceps and needle. We then pulled apart the anterior and posterior body sections using forceps, with the result that the ovaries remained intact with the posterior section, to be then preserved in Kahle's solution. Mature oocytes were dissected at a later date. Although this procedure has been used successfully with other bee taxa, the results proved only partly satisfactory in that the chorionic microstructure of most dissected mature oocytes was not fully developed when compared with that of eggs that had already been deposited. For example, the mature oocyte of *Radoszkowskiana rufiventris* showed only a single pore in the micropylar area, whereas the chorion still covering the first instar head of the same species shows surface sculpturing (fig. 32) similar to that on the oocytes of *Coelioxys coturnix* (fig. 33) and the egg chorion of *M. nigripes* (fig. 34). Similarly, mature oocytes of *Coelioxys decipiens* and *Megachile nigripes* also lacked surface sculpturing. Only the mature oocytes of *C. coturnix* (fig. 33) appeared to be fully developed. Either our sampling of dissected mature oocytes was inadequate, or the chorionic microstructure of the micropyle is deposited just before oviposition.

Descriptions of mature larvae are based on postdefecating forms, with significant differences of predefecating larvae noted in a separate paragraph following the main description.

We report on a number of diagnostic differences in the larval labrum among the taxa treated here. In all cases, the labrum is short and broad and terminates in a broadly emarginate apex. The differences among the taxa pertain to the shape of the labral sclerite and to the pigmentation of the labrum. The labral sclerite stretches across the base of the labrum, and on the lateral extremes it curves downward before ending. It is connected to the front of the head capsule by a membranous area, which is unpigmented whereas the sclerite is more or less pigmented, so that when viewed on untreated head capsules, its upper margin is usually quite discernable, as can be seen in figure 43. The shape of its upper margin is an important feature whereby, for example, the larva of *Radoszkowskiana rufiventris* (fig. 43) can be distinguished from that of *Megachile nigripes* (fig. 46). Interestingly, this sclerite, so obvious on an untreated head or on a head that has been cleared in an aqueous solution of sodium hydroxide, is completely invisible when viewed with an extended variable-pressure scanning electron microscope (SEM) (fig. 92).

Apical to the labral sclerite, the labrum of mature larvae is typically membranous and beset with numerous tuberculate sensilla, which in some cases are pigmented and thus obscure the lower margin of the labral sclerite unless the head capsule is cleared. In fig-

ures 43–46 we have artificially identified the lower boundary with a dashed line. In the case of the two species of *Coelioxys* (figs. 44, 45) this membranous area is the site of a very darkly pigmented median spot when viewed on an uncleared labrum, but this pigmentation is partly lost when viewed on a cleared specimen.

Specimens of eggs/oocytes and larvae studied with the Hitachi S-5700 SEM were first critical-point dried and coated with gold/palladium. Postdefecating larvae so treated were subjected to considerable shrinkage and distortion, resulting in flaking of the external coating of the exoskeleton. This coating may be a dried secretion that helps with water conservation during hibernation. In addition, the treatment also causes the salivary lips to open, revealing details of microstructure that have rarely been described before for any bee larva. (Clearing specimens in an aqueous solution of sodium hydroxide removes the secretion but further distorts the cuticle.) As can be seen in figure 92, a micrograph taken with the EVO SEM shows little distortion of the exoskeleton, and the salivary lips remain closed; therefore, the internal microstructures (figs. 94–98) are unrevealed.

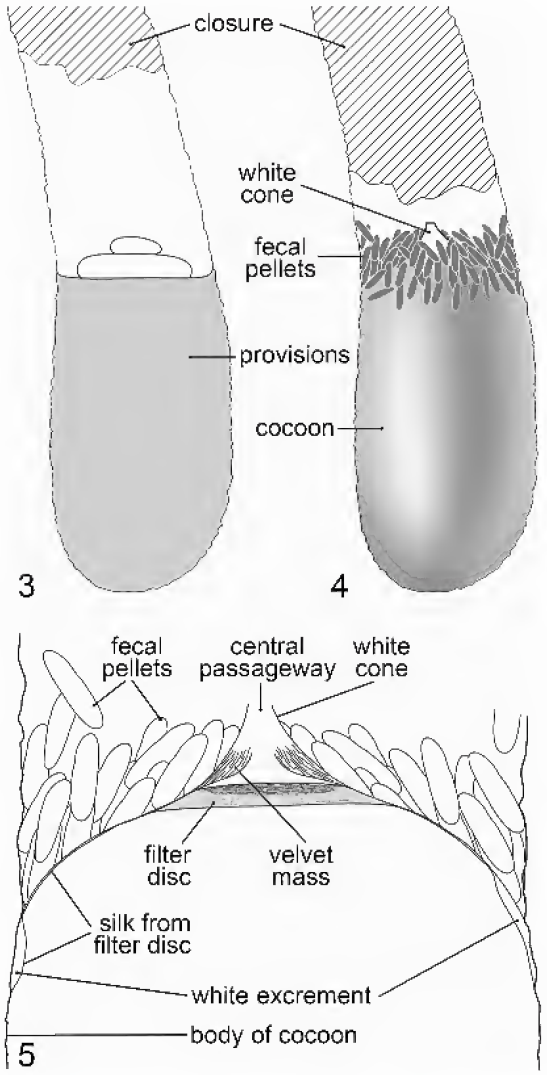
The upper and lower lips each seems composed of flat, overlapping tiles that end abruptly near the outer surface of the lip, so that one can see their overlapping arrangement (figs. 78, 79, 82, 86, 88, 91, 95, 96, 98). These tiles fuse with one another at their bases. Their distal edge may be truncated, curved, or finely scalloped. However, with tiles ending before the leading edge of the lip, the distal margin is often papillate (e.g., figs. 96, 98). Farther into the salivary duct, the distal edge may take on the appearance of a linear series of papillae without the rest of the tile being defined (e.g., figs. 86, 96). Still farther in, the salivary duct often bears pronounced parallel ridges (e.g., 91). We think it likely that channels formed by the ridges serve to extrude a band of viscous silk evenly during cocoon spinning and that the tiled leading edge of the lips serves to make them flexible. Exactly how the cocoon-spinning larva can extrude fine strands of silk in one area of the cocoon and broader ribbons of silk in another is unknown. We do not know what effect, if any, that the different microstructures of the spinning apparatuses of the various species have on the construction of their cocoons.

DESCRIPTION OF NEST OF *MEGACHILE (PSEUDOMEGACHILE) NIGRIPES*

The following is a brief description of the nest of *Megachile nigripes,* the host bee of both *Radoszkowskiana rufiventris* and *Coelioxys decipiens*. Nests were shallow tunnels in the vertical surfaces of hard, dry, "mud" walls, so that entire nests penetrated the wall to a maximum of about 8 cm. We did not attempt to excavate single nests to analyze their architecture because the emphasis of the investigation concerned the biology of *R. rufiventris* to which only the structure of the host cell was primarily pertinent. However, we noted that the main tunnels entered the verticals wall and descended obliquely. Most cells of about the same age appeared to be arranged closely, sometimes almost contiguously, suggesting that they represented the nest of a single female.

Cell orientation was more or less vertical, with the rear of the cell lower than the upper end (or closure). Cells seemed to be arranged singly, that is, not in linear series, but this should be confirmed. In shape, they were more or less elongate, varying in length from 16 to 21 mm (see figs. 3, 4), but they were quite uniform in maximum diameter, which was always 6–7 mm. The variability in length results from females constructing the closures at different levels along the tunnel leading to the cell. The cell's long axis was slightly curved, particularly noticeable on long cells. At the entrance, cell diameter was 5 mm (but about 13 from the bottom of the cell), which gradually increased to a diameter of 6 mm or a bit more. The lower (rear) end of the cell was rounded. Thus, in lateral view, a long cell is not only elongate but almost parallel-sided (figs. 3, 4).

The internal cell surface was considerably darker than the substrate elsewhere, a darkness that bled into the cell wall for a distance of about 0.5–1.0 mm. There was no indication that the cell wall was constructed in a larger excavation in that there was no demarcation



Figs. 3–5. Diagrams of cells and cocoon of *Megachile nigripes*, lateral views. **3.** Taller cell, showing provisions with egg of *Radoszkowskiana rufiventris* deposited on floating egg of host. **4.** Shorter cell with cocoon of *M. nigripes*. **5.** Close-up of cross section of upper end of cocoon of *M. nigripes*; for explanations, see text.

between the substrate and a wall of soil particles that might have been imported from the outside. However, large numbers of females were observed flying close over the ground, often stopping to collect soil from the horizontal paths and roadways next to the nesting walls. Such behavior may be for materials for cell closure, as mentioned below, but it also may play some other role in nest construction. The inner cell surface was uneven but not as coarse as the edge of the fractured substrate, suggesting that the female somehow works the surface. It appeared faintly shiny because of the thin coating of the dark substance that had a texture of a sticky wax when probed with fine forceps under a stereomicroscope. A faint pattern of fine striations at right angles to the long axis of the cell was detected on exposed cell surfaces. When tested with a water droplet placed on the cell surface, the droplet turned slightly cloudy but was not absorbed immediately, indicating that the cell surface was water retardant but not truly waterproof. When a piece of the wall with surface attached was submerged in water, the surface remained intact while the substrate beneath disassembled. When then touched with forceps, the surface immediately also disassembled, thus indicating a lack of structural strength that would have been present if the surface had been coated with a hydrophobic secretion. The source of the darkish coating is unknown, but we suspect it to be nothing more than very fine soil particles bound together by partly dried nectar.

Provisions were a sticky mass of pollen and nectar. When freshly stored, they occupied the lower 10–13 mm of the cell, and the eggs of *Megachile nigripes* floated on the surface film, as they nearly spanned the cell diameter (fig. 3). The cocoons of this species, as well as those of *Radoszkowskiana rufiventris* (and presumably *Coelioxys decipiens*), occupied the lower end of the cells, leaving a more-or-less long empty space between the top of the cocoon and the cell closure.

Cell closures (fig. 29) were invariably strongly convex on the inside, with their surfaces being coarsely textured because of sand grains that were occasionally larger than soil particles from the surrounding substrate. The closures were dark on their lower surface, presumably because of the same substance that coated the cell wall. Females of *Megachile nigripes* were observed crawling from the burrow entrances, chewing the surface of the vertical adobe walls, and then descending again into their nests. They were also commonly seen flying over the horizontal surfaces at the bases of walls and stopping momentar-

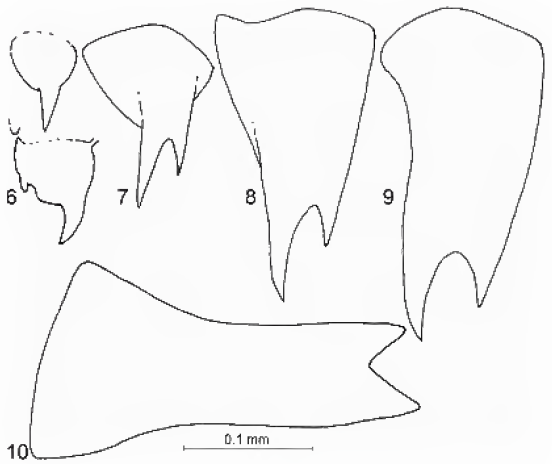
ily, presumably to collect soil particles, as mentioned above. We suspect that these actions may account for the material used in constructing closures and perhaps for backfilling tunnels leading to closures.

Nests of *Megachile nigripes* where we excavated appeared to be the main source of cavities in the wall. They were often reused by other individuals of the same species, as attested by provisions of a new inhabitant of the cell placed in the remnants of a previous year's cocoon. Cells of leaf-cutting species and of one or more small mason species were frequently found built into the cells of this species and even into vacated cocoons.

LARVAL DEVELOPMENT

Radoszkowskiana rufiventris, *Coelioxys decipiens*, and their host, *Megachile nigripes*, have five larval instars. We determined the number of instars by retrieving the cast larval exoskeletons ("skins") that accumulate one on top of the previous one under the instars as they molt and by carefully separating them under a stereomicroscope. Later instars can be identified easily because faintly pigmented head capsules and mandibles are observable on the skins, as are spiracles. First instars are poorly pigmented and small, but their head capsules (particularly the hypostomal ridges), mandibular apices (figs. 6–10), and spiracular atria and subatria can be detected when viewed with a compound microscope. We were unable to verify the number of larval instars of *C. coturnix* and its host.

In the cast first-instar skin of *Radoszkowskiana rufiventris*, the mandible seemed to consist of a single, curved, strongly sclerotized apical tooth. However, when the complete head capsule of a first instar was examined with an SEM (fig. 20), we detected that, basal to the apical tooth, the mandible possessed a second tooth (figs. 21, 22), which was less strongly sclerotized (and therefore initially overlooked on the cast skin) and which bore a number of long spines. The possible function of the second, spined tooth is discussed later under "Eclosion Process". Thus, the mandible of the first instar of this cleptoparasite is bidentate, as is that of the first-instar *Megachile nigripes.*



Figs. 6–10. Right mandibles of larval instars of *Radoszkowskiana rufiventris*, outer views, with apices drawn in maximum profile. **6.** First instar, above, outer view with second tooth eclipsed by apical tooth, and, below, ventral view showing basal tooth.**7.** Second instar. **8.** Third instar. **9.** Fourth instar. **10.** Fifth instar. All figures drawn to same scale.

Last instars can be recognized because of their projecting salivary lips and because they are the only instar that has long, conspicuous setae on its head and body.

Five larval instars appears to be the constant number for all Megachilidae (Baker, 1971; Rozen and Özbek, 2004; Torchio, 1989a, 1989b) and perhaps for all bees (Torchio, personal commun., 3 February 2004), although Alves-dos-Santos et al. (2002) reported that *Coelioxoides waltheriae* Ducke (Apidae: Apinae: Tetrapediini) has only four instars. This constancy is interesting because on theoretical grounds (van Valen, 1973) many cleptoparasites are in an arms race with the host immature; the latter must be eliminated while it is vulnerable before it depletes the provisions. One way that this might be accomplished is for the parasite to have fewer instars than the host, so that the parasite develops more rapidly than the host. We now know that species of *Stelis* (Torchio, 1989b), *Dioxys* (Rozen and Özbek, 2004), *Coelioxys* (Baker, 1971; Rozen and Kamel, 2006), and *Radoszkowskiana* (present study) have five instars. Thus, at least in the Megachilidae, reduction in the number of instars does not

seem to be an option for outracing the developing host.

NESTING[4] BIOLOGY OF *RADOSZKOWSKIANA RUFIVENTRIS*

The following account of the developmental biology of this species is based on only six specimens, four of which were initially encountered as eggs and two of which as early instars. We were able to follow their development intermittently as we tried to rear them. Information about the first instar, unless stated otherwise, is based on a single specimen preserved while attached to the host egg. Our observations, therefore, were fragmentary.

Egg Deposition: The first egg of *Radoszkowskiana rufiventris* was discovered on May 17, 2004, resting on top of an egg of *Megachile nigripes* that was floating lengthwise on the surface of the provisions with its ventral surface partly recessed into the provisions (possibly resulting from being transported to the laboratory). The two eggs were only slightly misaligned from being parallel (fig. 11); the parasite egg was closer to one end of the host egg than to the other. Later, when the parasite egg hatched and the embryo of the host had developed, we determined that they were both oriented in the same direction. The parasite egg was slightly less than half the length of that of the host (see description of the egg/mature oocyte of *R. rufiventris,* below).

A year later, we discovered three more eggs of *Radoszkowskiana rufiventris* on eggs of *Megachile nigripes.* In each case, the parasite egg was approximately parallel to that of the host (fig. 12), and each parasite hatched a day later. In two of these cases, the parasite egg

[4]One reviewer suggested that the word "Nesting" should be deleted from "Nesting Biology" since cleptoparasites do not make nests. Although this is generally (but not invariably; see Iwata, 1933) true, they always use nests provided by other bees for egg deposition, larval feeding and defecation, cocoon spinning (for cocoon making species), and hibernation/aestivation, just as do nonparasitic bees. We therefore think that "Nesting Biology" is appropriately used here; it makes a distinction between biological activities that occur in nests from such other behaviors (especially mating behavior, although there are even exceptions to this) that generally do not involve nests.

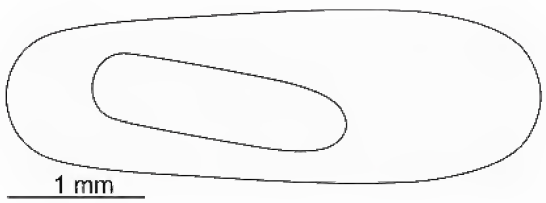


Fig. 11. Diagram of egg *Radoszkowskiana rufiventris* position on egg of *Megachile nigripes* found on May 17, 2004, anterior ends to left, dorsal view.

certainly pointed in the same direction as that of the host. Also, two first or second instars were found on eggs of the host species: one positioned so that it was parallel to and pointed in the same direction as the host; the second was also parallel to the host but relative directions could not be determined.

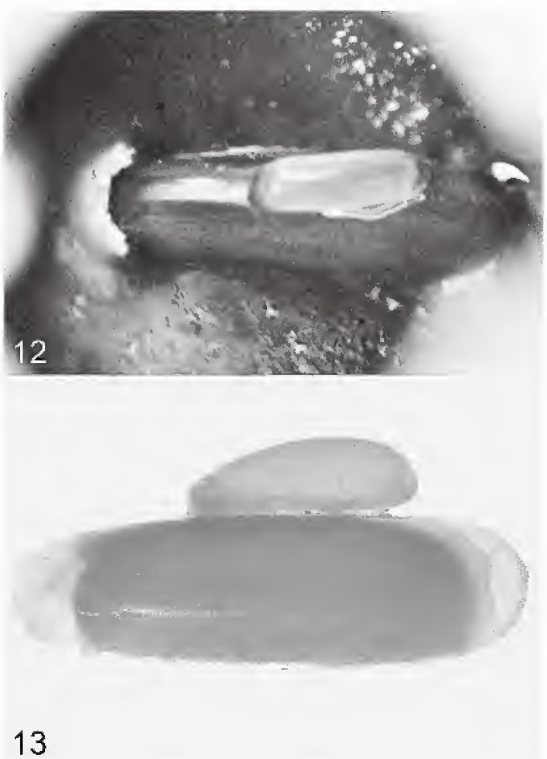


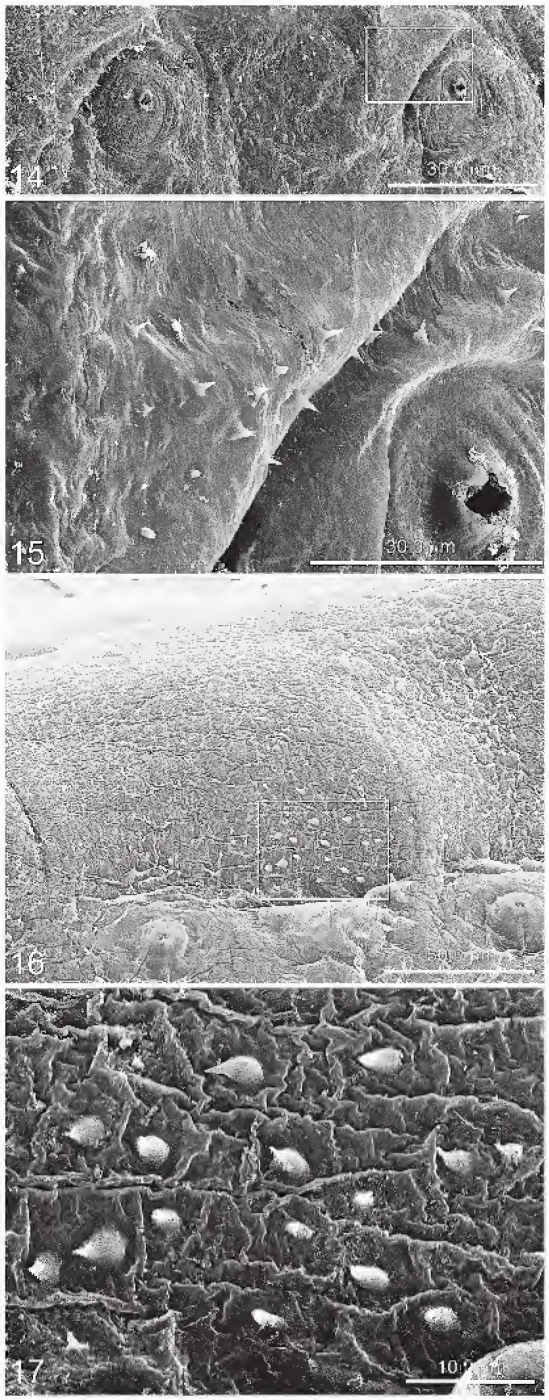
Fig. 12. Photograph of live egg of *Radoszkowskiana rufiventris* on top of egg of *Megachile nigripes*, collected on June 1, 2005, dorsal view. Fig. 13. Photograph of same individual of *R. rufiventris* preserved as pharate first instar with host egg on June 2, 2005, lateral view.

From these five findings as well as that from the original observation a year earlier, we conclude that the egg of the cleptoparasite is normally deposited on the dorsal surface of, and parallel to, the host egg, as far forward so that its anterior end is either over the head of the developing host embryo, over the midbody of the embryo, or perhaps even slightly behind the midpoint of the embryo. Whether the host and parasite eggs will always be found to point in the same direction must be confirmed by further observations. We did not find eggs or early instars of *R. rufiventris* elsewhere in host cells.

Because solitary bees close their cells immediately after oviposition, we concluded that females of *Radoszkowskiana rufiventris* either (1) open closed host brood cells to deposit their eggs on the host eggs or (2) attack host cells when the female *Megachile nigripes* are away gathering soil particles to construct cell closures. Both scenarios seemed plausible in the absence of further data. If the first were correct, we would expect to find a plugged opening in the cell closure through which the parasite female had introduced her tapering metasoma to place her egg on that of the host. Such filled openings have been documented for the distantly related *Protosiris gigas* Melo (Apidae: Apinae: Osirini) (Rozen et al., 2006: figs. 5, 6). The second scenario requires the parasite to find the host nest, enter it, and deposit its egg between the time the host deposits her egg and when she caps the cell, a presumably brief moment. A similar brief window of opportunity is known to exist in the case of the distantly related *Coelioxoides waltheriae* (Alves-dos-Santos et al., 2002) in which the host female seals her cell closure with imported soil, after which she adds floral oils to the closure that "becomes hard and becomes brittle in a few hours" to make the closure impenetrable to the insertion of the parasite egg. However, while the host female is away gathering additional closure material, the still soft closure is unguarded and vulnerable to parasite attack, the window of opportunity for *C. waltheriae.* After the *Tetrapedia* female applies the last application of floral oils to the closure of the outermost cell in a series, she often sits in front of the closure until the hardening process of the closure is completed. Discovery of an egg of *R. rufiventris* on the last day of the fieldtrip in 2005 was accompanied by recovery of the host cell closure. Because the egg bore no indication of having been penetrated by the female cleptoparasite, we tentatively conclude that the second scenario is correct.

Although we have no direct observations of females of *Radoszkowskiana rufiventris* ovipositing, the known facts suggest that after gaining access to the cell entrance, the female backs her long, tapering, extensible metasoma into the cell lumen and gently, probably with the apical brush of fine sensilla of her sixth metasomal sternum, identifies the fragile host egg and its direction on the provisions. Perhaps after first adjusting her body position so that she can orient her egg to coincide with that of the host, she then deposits her egg on the dorsal surface of the host egg and departs, no doubt in some haste because of the threat of a returning host.

Developmental Rates: The six specimens of immatures of *Radoszkowskiana rufiventris* that we were briefly able to rear provide some information on development rates and on larval behavior and anatomical changes. Although we have no complete record of the egg incubation period, the egg that we observed for the longest period was discovered at the nesting site during midday and hatched during the night following the next 2 days, or approximately 2.5 days later. In all four cases in which eggs of *R. rufiventris* were observed, they all hatched before the host embryo had fully developed despite the fact that the parasite eggs were deposited after the host egg. The duration of the first larval stadium is brief based on two individuals, perhaps only about 1 day. The duration of the second larval stadium is apparently longer, with three cases suggesting 2–3 days. One individual took 8–9 days from hatching to the start of the fifth larval instar, so that the third and fourth stadia probably lasted about 2.5 days each. We have no data regarding the total length of the larval feeding period. Because rearing specimens in cells that have been opened and subjected to unnatural conditions might influence developmental rate, data presented above are tentative.



Figs. 14–17. SEM micrographs of midbody segments of first instars of *Megachile nigripes* and *Radoszkowskiana rufiventris*, showing differences in spicules, anterior ends toward left, lateral view; for explanation, see text. **14.** Two midbody segments of *M. nigripes* showing sharp-pointed, thornlike spicules above spiracles. **15.** Close-up of area identified by rectangle in fig. 14. **16.** Two midbody segments of *R. rufiventris*. **17.** Close-up of area identified by rectangle in fig. 16.

Eclosion Process: A problem involved when describing eclosion of bee eggs concerns defining when an egg hatches: first instars of most bee taxa remain closely surrounded by the egg chorion (Alves-dos-Santos et al., 2002; Rozen, 1964, 1969; Rozen and Buchmann, 1990; Rozen et al., 2006; Rust et al, 1989; Torchio, 1989a, 1989b; Torchio and Trostle, 1986), although many (but not all) cleptoparasitic first instars immediately emerge from their chorions (see references in Rozen, 2003: table 1). For taxa with pharate first instars (i.e., those partly surrounded by chorion), hatching might be defined by when the chorion splits just above the spiracular line and the tracheae become filled with air. The appearance of a segmented body alone cannot be interpreted as the emergence of the first instar since segmentation becomes evident as the embryo ingests amniotic fluid prior to hatching and before the chorion splits and the tracheal systems fills with air.

With one individual of *Radoszkowskiana rufiventris*, we knew that the egg was about to hatch because the embryo had reoriented so that its dorsal surface was now facing the dorsal surface of the egg at 10:15 AM (DuPraw, 1967; Torchio, 1984). When we next observed it at 4:50 PM the same day, the amniotic fluid had disappeared (no doubt ingested), the tracheae were gas (air) filled, and slight motion of the head was detected. Thus, the first instar had eclosed. Eclosion of the three other eggs of this species was similarly detected by the appearance of air-filled tracheae associated with muscle activity in the head capsule. In none of the cases could we be certain how the chorion ruptured because of small body size and the lack of visual contrast between chorion and larval integument.

The egg hatching process of *Radoszkowskiana rufiventris* and of *Megachile nigripes* may be different from one another as indicated by the anatomy of the first instars. In the case of the host, a linear band of erect, minute, sharply pointed, thornlike projections

(figs. 14, 15) occurs along each side of the body just above the spiracular line. Similar bands of thornlike projections have been detected in first instars of other bee taxa, and Rozen et al. (2006) postulated that they serve as the tearing mechanism, assisting the chorion to split just above the spiracular line on each side of the body, allowing eclosion.[5] Those authors also showed SEM micrographs of the erect, sharply pointed, thornlike projections on the first instar of *Monoeca haemorrhoidalis* (Smith) (Apidae: Apinae) (Rozen et al., 2006: figs. 14, 15) similar to those of *Megachile nigripes*. The similarity of these projections in different families suggests that they are homologous. They are probably involved with the eclosion in all cases where chorions split laterally along the spiracular line, which is known to occur in Apidae and Megachilidae and probably in other families as well.

SEM examination of the first instar of *Radoszkowskiana rufiventris* revealed that the bands of erect, thornlike projections were either modified or replaced. Instead, there is a patch of peculiar spicules centered somewhat above and posterior to each spiracle (figs. 16, 17). The spicules in these patches are small and sharply point and are strongly directed anteriorly. Their function is unknown but is possibly associated with the shedding of the chorion. Spicules of any sort were absent elsewhere on the parts of the body not covered by chorion.

Hospicidal Mechanism and Larval Feeding Activities: The first instar of *Radoszkowskiana rufiventris* attached to the host egg (fig. 13) was preserved in Kahle's solution, to be examined later with an SEM at the American Museum of Natural History. Just before it was prepared for SEM study, the first instar and the host egg had to be pried apart with forceps, with the only point of attachment being the parasite's mandibles that were securely clamped onto the host chorion. Both the host egg and the first instar were then

[5]DuPraw (1967) identified a "hatching enzyme" of unknown source to be responsible for the dissolution of the chorion along the spiracular line in honeybees. If, as it seems likely, such an enzyme is involved with eclosion in other bees, then hatching is not just a mechanical tearing of the chorion, but a combination of some chemical and mechanical interaction.

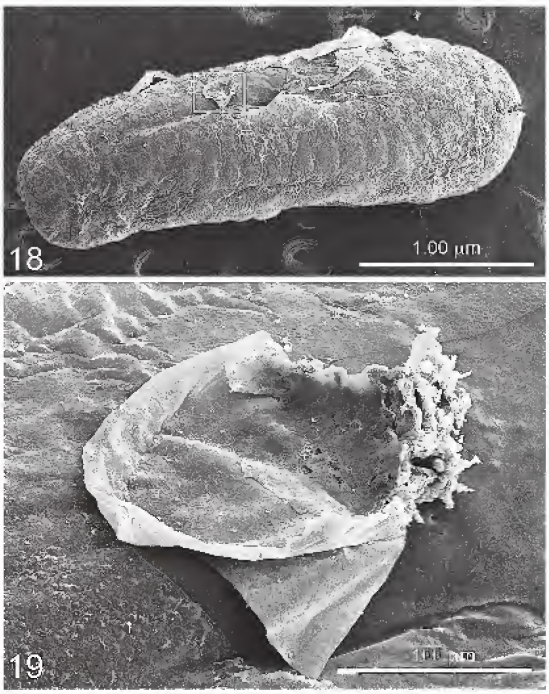
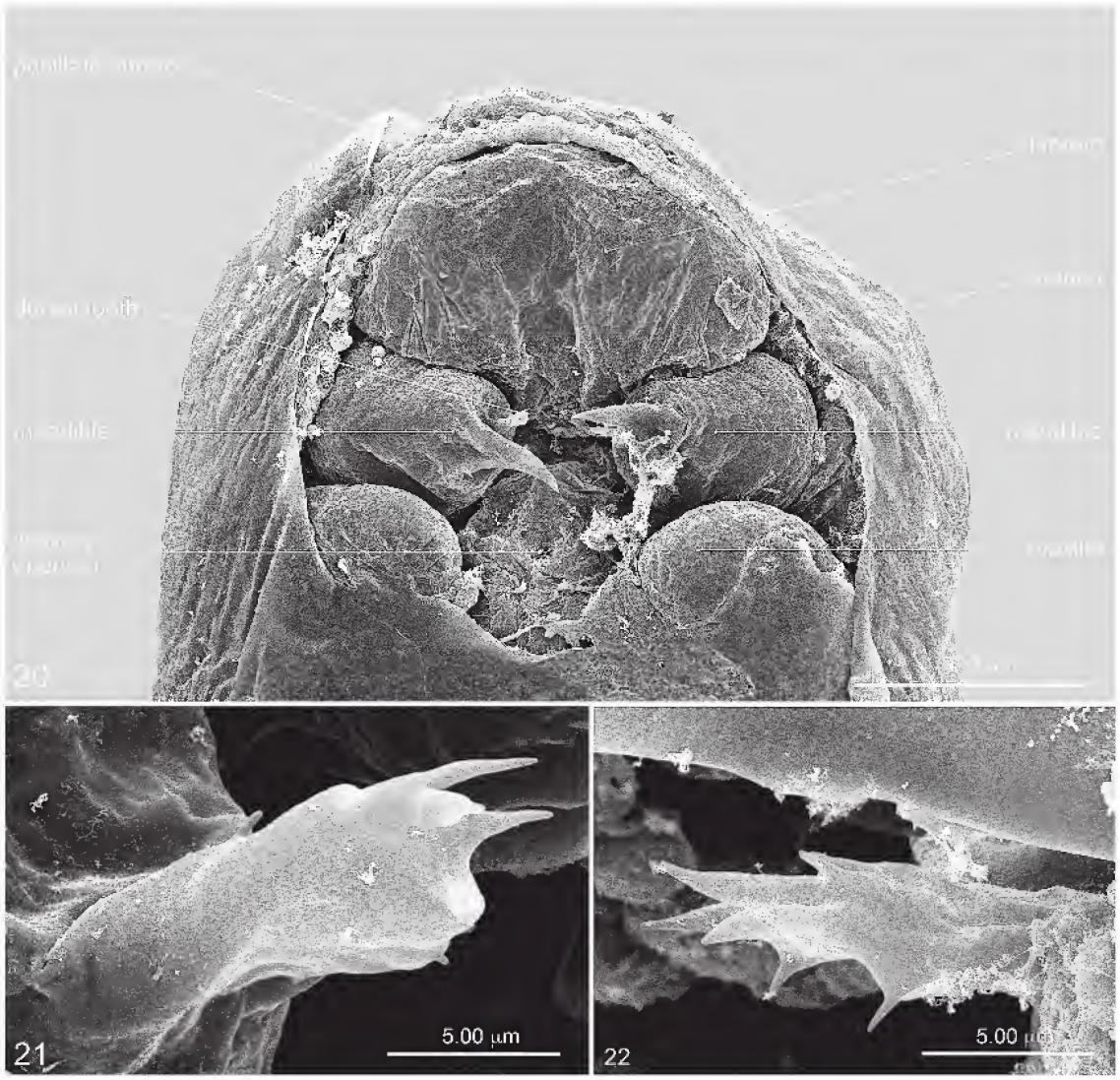


Fig. 18. SEM micrographs of egg of *Megachile nigripes* from which first instar of *Radoszkowskiana rufiventris* had been removed, leaving behind piece of chorion that had covered its mouthparts, identified by rectangle. Fig. 19. Close-up of piece of chorion of *R. rufiventris* identified by rectangle in fig. 18.

examined with an SEM. The resulting micrograph of the host egg revealed that a small piece of the first instar's chorion that covered its mouthparts had ripped away when we separated the cleptoparasite from the host egg and remained on the egg. A close-up of the head chorion (fig. 19) revealed a number of small rents in the head chorion that may have been made by the first instar's mandibles. These facts suggest that the first instar may bite through its own chorion and the chorion of the host and commence to feed on the host's yoke, which is possibly drawn through the small tears in the chorions of the host and parasite by the sucking action of the parasitic first instar. Alternatively, the cleptoparasite may make a larger opening in its chorion with its mandible and then rupture the host chorion to feed on its yoke. In any event, it seems clear that the first instar of *R. rufiventris*, while pharate within its chorion, attacks the host egg and starts to feed on it.



Figs. 20–22. First instar of *Radoszkowskiana rufiventris*, ventral view. **20.** Anterior end. **21, 22.** Close-up of upper tooth of right and left mandible, respectively.

Figure 20 is a close-up in ventral view of the anterior end of the first instar of *Radoszkowskiana rufiventris* from which the piece of chorion had been torn away when host and parasite were separate. The peculiar stratum with a papillate outer surface covering the front of the labrum cannot be explained at this time and warrants further investigation to reveal its anatomy and function. Of special interest are the mandibles. Although, as stated above, we thought at first that each had a simple, curved, sclerotized apex, we discovered from the SEM micrographs that each possessed a more basal, less sclerotized, second projection (figs. 21, 22) that we interpret to be the upper tooth, which bears numerous spines. The function of this tooth with its spined apex is unknown, but possibly these teeth on their respective mandibles work opposite to one another to pull taught the chorion between them as the mandibles are closed, thus stretching and stabilizing the chorion so that the two apical, fanglike teeth can pierce the chorion. The spine-bearing

apices appear as if they might provide the necessary purchase to prevent the chorion from slipping.

The host egg is obviously a potential future competitor of the larva of *Radoszkowskiana rufiventris* for the stored food, and therefore it must be destroyed. However, we do not know if the host egg is also nutritionally important for the development of the parasite, if it is a necessary source of water for the young and therefore small parasite, or if it is important for both reasons. Linsley and MacSwain (1955) observed the first instar of *Nomada opacella* Timberlake (Apidae: Nomadinae) "feeding" on the host egg, presumably implying that nutrition was involved. On the other hand, Alves-dos-Santos et al. (2002) have suggested that the water content of the host egg may be important for the first instar of *Coelioxoides waltheriae* because its egg is small by comparison with that of its host, which lives in an environment where desiccation could be a problem. A test of the general hypothesis that feeding/drinking is important might be to try to rear the parasite on provisions where the host egg has been manually removed before the parasite egg has hatched.

On hatching, young larvae of *Radoszkowskiana rufiventris* immediately chew into the host eggs beneath their heads without moving from their oviposition placement and start ingesting (sucking) yolk material (fig. 23). They slowly become physogastric, with their bodies gradually swelling for a day or so, extending backward, and filling with nearly clear liquid from the host egg, without traces of yellow pollen. The host eggs slowly become depleted. Although chewing or sucking motions of the cleptoparasite head are sometimes evident, the mouthparts are continuously held against the same point on the host egg; the cleptoparasite does not remove its head to bite repeatedly into the host as if to attack and kill the host, as is the case with second or third instars of *Coelioxys*.

With two individuals, feeding on the host eggs continued partway into the second larval stadium, and, as second instars, they completely depleted the host eggs, which were then reduced to depressions on the surface of the provisions, with their shiny chorions left behind identifying the depressions (fig. 24). The second instars were able to extend and turn the anterior parts of the body to one or the other side of the former host to commence feeding on the provisions while the posterior part of the body remained in place. The yellow color of the pollen became visible through their transparent cuticle and body tissue. Subsequent instars continue the feeding process (figs. 25, 26), and their mandibular anatomy changes from one instar to the next (figs. 6–10).

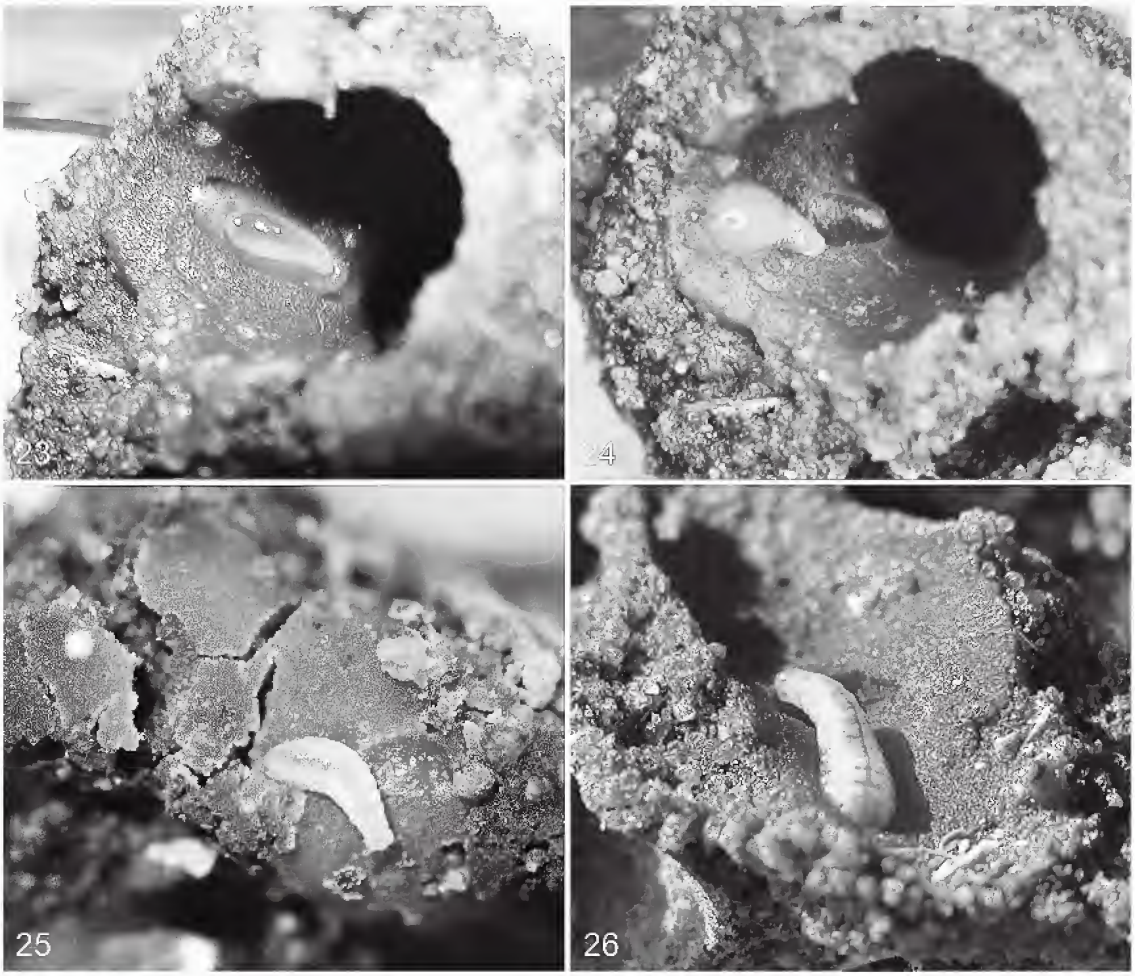
There is no indication that the early instars of *Radoszkowskiana rufiventris* are capable of crawling; the young larva is inactive and has no apparent mechanism for crawling over the surface of the sticky provisions. Once it starts consuming the host egg and then the provisions, its physogastric body does not lend itself to moving. The anatomy of the first instar with hypognathous head and curved, short, but sharply pointed, apically sclerotized (and pigmented) mandibles would appear to be adaptations for killing only the passive host egg.

Defecation and Cocoon Spinning: Larvae of both *Megachile nigripes* and *Radoszkowskiana rufiventris* commence defecation at the beginning of their last (fifth) larval stadium, and it would be surprising if that were not also true for *Coelioxys decipiens*.

The cocoons of *Radoszkowskiana rufiventris* (fig. 27), *Coelioxys decipiens*, and *Megachile nigripes* (fig. 28) are of similar size and shape. However, they can be identified not only by the immature stages within but also by the shape and color of their nipples and the thickness of the cocoon fabric, as identified in the following descriptions. As noted below, many cocoons were built into the remnants of cocoons from previous generations.

We excavated only two dry cocoons of *Radoszkowskiana rufiventris* (fig. 28) from the hard "mud" walls; later, many were washed from wall material that was disassembled with water, and they were immediately preserved in ethanol. The dry cocoons had a shiny outer surface that we later realized was actually from remnants of the host cocoon from a previous generation.

The specimens preserved in ethanol provided the clearest understanding of the construction and structure of the cocoon of this



Figs. 23–26. Photographs of feeding larval instars of *Radoszkowskiana rufiventris*. **23.** First instar three days after collection as egg, on partly consumed host egg. **24.** Second instar of same individual with opaque, yellowish abdomen, two days later, and chorion of completely consumed host egg. **25.** Probably third instar, different individual; note shiny chorion of host egg to the left of its forebody. **26.** Fourth instar of same individual as in figs. 23, 24, nine days after being collected as egg; two days later, larvae became fifth instar.

species. However, with all such specimens we discovered that the fabric consisted of layers of silk that had more or less separated because of the preservative. On the two dry specimens we saw that the layers were actually closely appressed to one another with some flattened fecal pellets sandwiched between the silken layers. Thus, the body of the cocoon was composed of a thick, leathery, single layer of material, far tougher and more opaque than the cocoon fabric of either *Megachile nigripes* or *Coelioxys decipiens*. A few specimens preserved in ethanol were encased in the remains of host cocoons from the previous generation. Fecal material from the cleptoparasite occurred between the old cocoon and the new one, indicating that defecation commenced before the parasite constructed its own cocoon, as already proven by our observation of the early last instar beginning to defecate while feeding. Other outer layers of silk woven later also contained fecal streaks, implying that defecation continued with silk production. We doubt, however, whether the total accumulated feces accounted for entire meconial load of the larva, and indeed on several specimens built into old cocoons, we detected a thick layer of feces under the rim of the old cocoon,

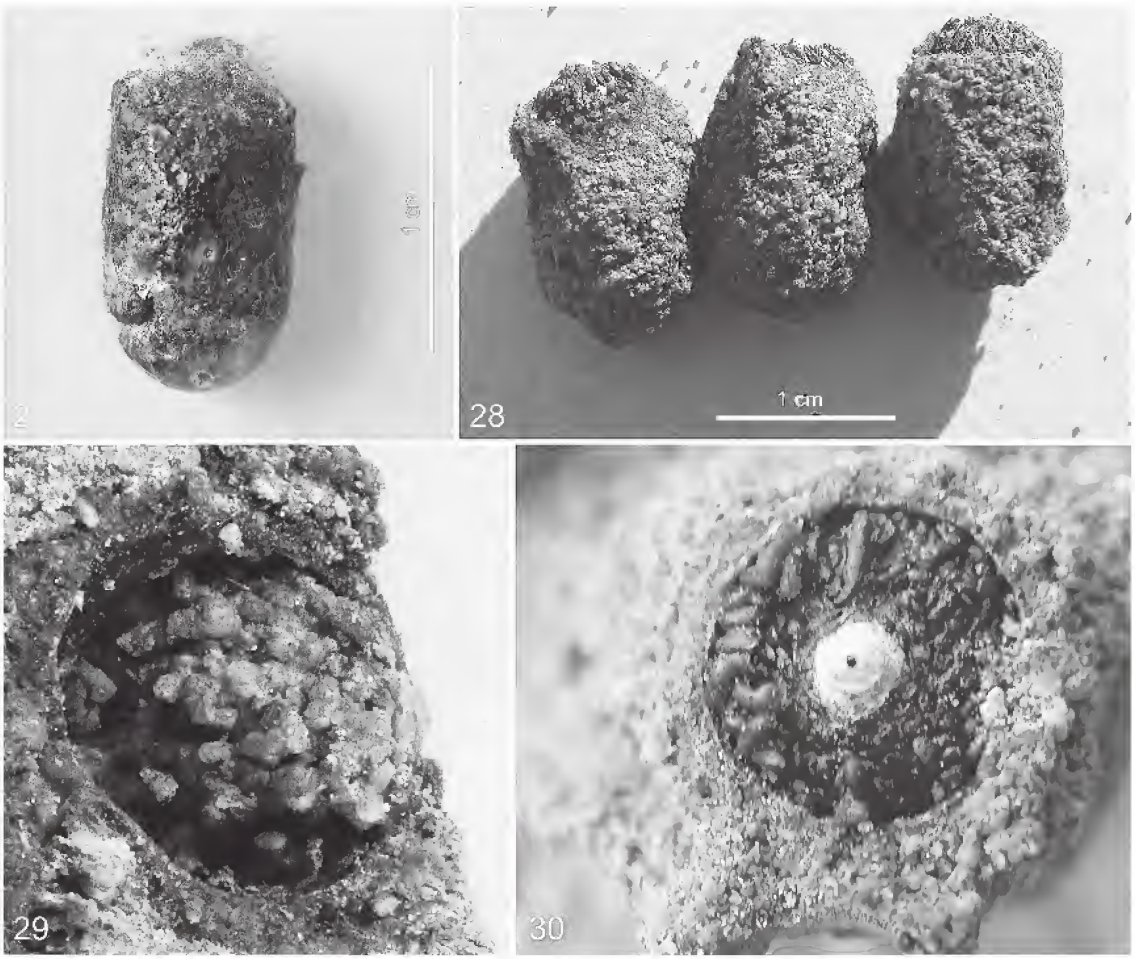


Fig. 27. Cocoon of *Radoszkowskiana rufiventris*, collected dry, lateral view; shiny surface resulted from its construction in remnant of cocoon of *Megachile nigripes* from previous generation. Figs. 28–30. *Megachile nigripes*. **28.** Three cocoons, lateral view, encased in material from cell wall; note feces and white nipples; nipple of cocoon on right accidentally destroyed. **29.** Close-up of cell closure as seen from inside cells. **30.** Outside, upper surface of cocoon showing dark fecal pellets and white nipple with central opening, top view.

suggesting that a larger quantity, washed away during extraction, had been deposited before the top of the front end of the cocoon of *Radoszkowskiana rufiventris* had been woven.

Inner layers of cocoon silk were free from feces, and these layers were normally longitudinally wrinkled on specimens that had lost the outer layers of silk during washing. We assume that the water bath had softened the feces, permitting the outer cocoon layers to remain behind. All silken layers were uniformly parchmentlike and loosely held together by fine silk fibers.

The reddish-brown, nippled upper end of the cocoon of *Radoszkowskiana rufiventris*, with an outside diameter of 5.0 mm, is domed with an external surface of wooly silk. The nipple itself is obtusely rounded, little elevated, and, in contrast to the nipple of *Megachile nigripes*, lacks an obvious opening. Internally on the periphery it is semitransparent and coated with a continuation of the sheet of nonfibrous silk. Near the center of the top there is a translucent disc of dense fibrous silk, about 3 mm in diameter and 0.5 mm thick at the center. This disc corresponds to the nipple

associated with other megachilid cocoons (see descriptions of cocoons of other species below) even though its external shape is evenly curved with the rest of the top (front end) of the cocoon. Its function presumably permits interchange of gases while excluding parasites and predators. In cells with the outer layers intact, the distribution of feces can be observed as opaque smears covering all surfaces including the top, with the single exception that none obscures the nipple disc. One cocoon was about 12 mm long and 6 mm in maximum diameter; others were as much as 14 mm long, and all had the maximum diameters of the cells.

Although cocoons of *Coelioxys decipiens* are similar in external appearance to those of *Radoszkowskiana rufiventris* because of their opacity and color, their fabric is thinner. The tops of the cocoons possessed a broad nipple, giving a domed, external shape to the entire top of the cocoon. The external surface of the nipple was fibrous. In cross section, the filter (0.4–0.7 mm thick) stretched across of the nipple immediately below the outer surface; internally, the filter formed a flat disc, 2.3–3.0 mm in diameter, with a texture seemingly identical to the cocoon filters of *R. rufiventris* and *M. nigripes.* The sides and bottom of the cocoon of *C. decipiens* were darkish brown, with a denser opaque appearance. With a nodular external surface, the fabric of all but one cocoon, when viewed in cross section, consisted of two layers of sheetlike silk, with the outer one being the remnant of a host cocoon and the inner one being deposited by the cleptoparasite, between which was abundant fecal material distributed throughout the cocoon except for the filter area. The cocoon fabric of this cleptoparasite is much thinner and weaker that the tough, multilayered fabric of the cocoon of *R. rufiventris.* In one cocoon, fine sand grains were scattered in the feces between the two silk layers. In another cocoon, the sand was more abundant and consisted not only of fine particles but of coarser grains as well. However, other cocoons were without sand. The one exception to the cocoon of this species consisting of two silk layers was a cocoon lacking the remnant of a host cocoon; it was a single layer of silk coated with feces on the outside. The inner surface of the cocoon of *C. decipiens* is a rather reflective brown.

Cocoons of *Coelioxys coturnix* and of its host, *Megachile minutissima*, are so similar that they cannot be reliably distinguished. Both are encased in leaf snippets that form the cell lining, and their shape varies depending on the cell dimensions. Most of the feces are piled at the front end of the cocoon, although some smear the outer surface of the cocoon and the leaf snippets. When the leaf snippets and feces are removed (e.g., by being soaked in ethanol), the cocoon wall is nearly transparent, clear to faintly tan, with faintly fibrous textures, except for the front end, where it is nearly opaque with a nipple composed of tan to reddish, fibrous silk. The nipple may be slightly elevated on the out-curved surface, but it often is slightly raised at the periphery, with its central part being faintly concave. With both species the diameter and perhaps degree of protrusion of the nipple vary, probably depending on the cell length—broad but low nipples on short cocoons, narrow but projecting nipples on long cocoons.

Because the cocoon of *Megachile nigripes* is better understood than those of *Radoszkowskiana* and *Coelioxys decipiens*, it is treated more fully herein. While still feeding and before spinning, the fifth instar of *M. nigripes* places its feces as dark, elongate pellets (figs. 4, 5, *fecal pellets*) around the cell wall 12–14 mm from the cell bottom. In doing so, it leaves a narrow, open central passageway to the cell above. In the cell below the feces, it then spins its cocoon.

The upper end of the cocoon of *Megachile nigripes* (fig. 5) is complex, constructed against and adhering to the lower end of the mass of fecal pellets. At its center is a narrowly pointed, nearly white, cone-shaped nipple, which rises through and beyond the central passageway formed by the dark feces. The larva first spins the outer surface of the nipple (fig. 5, *white cone*) as a thin, almost transparent fabric, leaving the apex of the nipple open. Thus, most of the visible part of the nipple appears white from the outside, unlike the broad, obtusely rounded, brownish nipples of cocoons of *Radoszkowskiana rufiventris* and *Coelioxys decipiens*. On fresh specimens (figs. 28, 30), the white nipple contrasts with

the surrounding very dark feces, but on specimens preserved in Kahle's solution, the feces pale to become nearly white. The fibers of this silk, though indistinct, seem to be applied concentrically, that is, ringing the nipple.

The larva then applies a thick, brown layer of silk to form the top of the cocoon. This layer is dense except where it approaches the central passageway (fig. 5, *central passageway*), at which point the numerous silk strands of the layer separate and end before they reach the central opening. Thus, the lower part of the nipple is ringed by a velvet mass of fine silk strands (fig. 5, *velvet mass*) that cause the nipple to have a dark base, as seen from the outside. Up to this point, the central passageway of the nipple is open, continuous with the lower part of the cell. However, the larva then spins a layer of fibrous silk across the passageway, resulting in a central flat disc (*filter disc*) of dense, fibrous silk about 2.5–3.0 mm in diameter and about 0.3 mm thick at the center. We assume that this disc functions as a filter to allow exchange of gases between the cocoon and the cell above and to exclude parasites and predators from attacking the immature bee within. The silk that forms this filter disc extends outward over the inner surface of the cocoon's upper end as a clear, nonfibrous sheet that indistinguishably fuses with the rest of the cocoon wall just below the top of the cocoon to form the presumably single silken layer of the body of the cocoon. The presence of thin blotches of pale, nearly white, fine-grained fecal material (fig. 5, *white excrement*) (on dry specimens contrasting with the dark brown feces above) between the two silk layers around the crown of the cocoon indicates that the larva voids this material during cocoon construction well after discharging the dark feces. We interpret this material to be the same as the final excrement (possibly excreted from the Malpighian tubes) that we have often seen in cells of other bees. It is unknown whether the lower, main part of the cocoon is constructed before the filter-disc part of the top of the cocoon is constructed or whether the entire top of the cocoon is constructed first and then the lower part is subsequently constructed. The length of the entire nipple from the inner surface of the disc to apex is approximately 1.2 mm.

The main body of the cocoon (i.e., all but the upper end) is composed of nearly transparent, nonfibrous, brown silk that so closely adheres to the cell wall that it cannot be easily separated from it. Because of the close bond between the cocoon and hard cell wall, the two elements together form a barrier against predators and parasites, and indeed some of the pebbles from the cell wall adhered to the cocoon when we removed it from the substrate.

VOLTINISM: *Radoszkowskiana rufiventris, Coelioxys decipiens,* and their host, *Megachile nigripes*, have a single generation a year that coincides with the flowering of *Trifolium alexandrinum*. All live immatures collected in mid-February were postdefecating forms in their cocoons. Pupation started shortly before S.M.K. visited the site in early April 2005.

RATES OF CLEPTOPARASITISM

On February 12, 2005, S.M.K. sampled the overwintering larvae at the nesting site that resulted in an estimated count of 720 larval specimens that could be identified as *Megachile nigripes*, *Radoszkowskiana rufiventris*, and *Coelioxys decipiens*. Twelve larvae (1.67%) were *R. rufiventris* and three (0.42%) were *C. decipiens*. On December 27, 2005, S.M.K. collected another sample estimated at 3,050 cocoons, which contained both live and dead individuals from the current generation, dead individuals from previous generations, and bombyliid larvae. Based solely on cocoon identifications (not cocoon contents), the rate of parasitism for *R. rufiventris* was 2.48% and that of *C. decipiens* was 0.74%. These appear to be extremely low rates of parasitism relative to the abundance of host and cleptoparasitic females flying along the vertical mud walls. This illusion can probably be explained in two ways: (1) Most host females were out of sight, either in their nests or foraging at flowers during our observations. (2) Cleptoparasitic females, at least those of *R. rufiventris*, may have a narrow window of opportunity as to when they can oviposit. According to one scenario (see "Egg Deposition", above), a *R. rufiventris* female must spend a considerable amount of time finding an open host cell in

which the host egg has already been deposited; when she does find such a cell, she must deposit her own egg and quickly depart before the host female returns. Hence, cleptoparasite females are rarely out of sight at the aggregation.

PRELIMINARY DESCRIPTION OF MATURE LARVAE OF THE MEGACHILINI

Because we treat larval exemplars of all three genera currently included in the Megachilini (Michener, 2000), we present the following description of the features that they hold in common, thereby eliminating unnecessary repetition in the descriptions of the species. It is obvious that these features are not diagnostic for the tribe, since most apply to all known larvae of both subfamilies of the Megachilidae (e.g., Grandi, 1961; Michener, 1953; McGinley and Rozen, 1987; Rozen, 1966, 1967, 1970, 1973a, 1973b, 1977, 1987; Rozen and Özbek, 2004).

Head: Integument generally colorless but following areas more or less brownish: antennal papilla, hypostomal and pleurostomal ridges, cardo, stipes, articulating arm of stipes, labral sclerite, mandibular ridges and apex, premental sclerite except becoming less pronounced below level of labial palpi, maxillary and labial palpi, and apex of salivary lips. Cranium with widely scattered, often long, setiform, tapering sensilla; sensilla of maxillary and labral apices mostly long, conspicuous; head integument without spicules, including lateral lobes of hypopharynx.

Head size small compared with body; head capsule much wider than long in frontal view. Tentorium complete, robust; anterior tentorial pit slightly closer to anterior mandibular articulation than to antennal papilla; posterior tentorial pit well impressed, in normal position at junction of hypostomal and postoccipital ridges. Median longitudinal thickening of head capsule weak, evident only near posterior margin of head. Postoccipital, hypostomal, and pleurostomal ridges well developed; hypostomal area tuberculate or not; epistomal ridge well developed laterad of anterior tentorial pit, absent between pits. Parietal band weak, scarcely evident. Antennal prominence weakly developed; antennal disc differentiated from papilla; papilla length substantially greater (apparently two or more times greater) than basal diameter, with approximately three sensilla. Front of head capsule in lateral view (figs. 47, 48, 83, 89) sloping normally so that labrum in line with or extends beyond clypeus, and clypeus beyond frons. Labrum (figs. 43–46) moderately wide compared with very short median length as seen in frontal view, its apex broadly emarginate, usually unsclerotized and bearing numerous sensilla; labral sclerite variably shaped and more or less pigmented but always evident; epipharyngeal surface without spicules.

Mandible (figs. 35–42) moderately short, robust, with two broad apical teeth, so that mandibular apex almost parallel-sided before abruptly ending when seen in inner view (figs. 36, 38, 40, 42), not tapering gradually to pointed apex; shape of apical teeth variable; when viewed dorsally (figs. 35, 37, 39, 41) or ventrally, mandible gradually tapering apically; inner apical surface with apical concavity shallow to indistinctly developed; cuspal area not developed, without denticles; outer surface with one or two setae sometimes borne on faint tubercle near base. Labiomaxillary region moderately strongly projecting in lateral view (figs. 47, 48). Maxillary apex curving mesad, so that palpus subapical; galea not evident although long setae present distad of palpus; palpus moderately long, approximately equal in length to antennal papilla; cardo and stipes well developed; articulating arm of stipes broad, darkly pigmented. Labium divided into prementum and postmentum; premental sclerite well developed, circling prementum, dorsal part narrow, darkly pigmented, lower part broad, weakly pigmented to scarcely noticeable; apex of labium normally narrow to exceedingly broad; labial palpus apparently slightly smaller, equal to, or longer than maxillary palpus. Salivary opening a wide transverse slit on projecting lips of variable breadth. Hypopharynx nonspiculate, not projecting, each side with lateral lobe separated by broad valley ascending directly to esophagus.

Body (figs. 49–52): Integument of postdefecating larva finely wrinkled (figs. 49–52); much of body with conspicuous, tapering, moderately long setae arising from conspicuous alveoli;

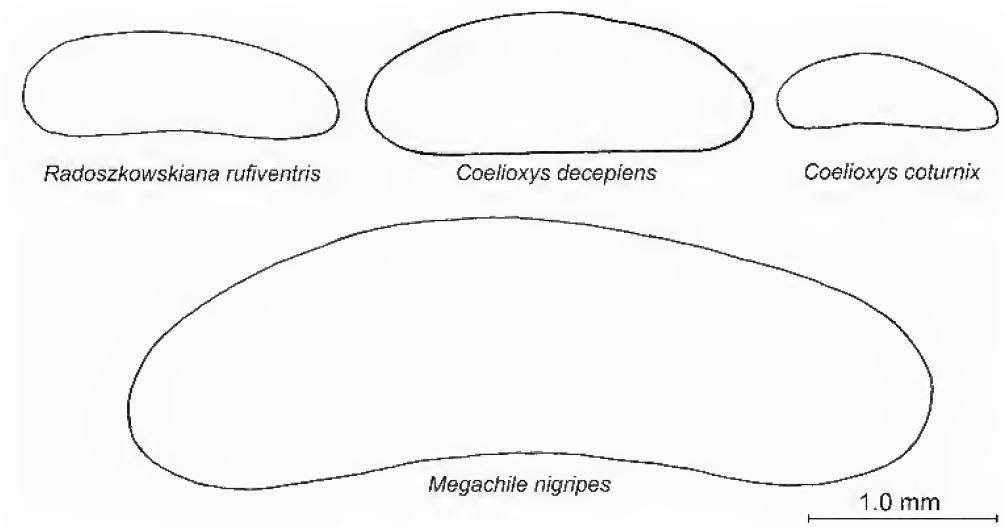


Fig. 31. Mature oocytes drawn in lateral view to same scale, anterior ends to the left.

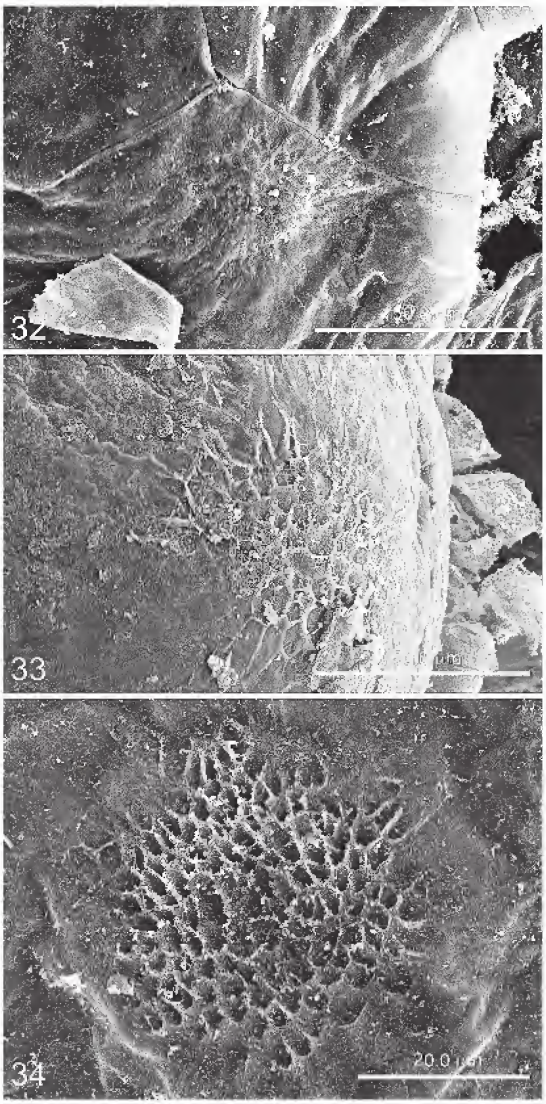
body with patches of fine, inconspicuous spicules. Body form robust, with widest part of body toward rear; intersegmental lines weakly incised; dorsal intrasegmental lines (separating cephalic and caudal annulets) sometimes nearly absent on uncleared specimens because of soft integument compressed by confines of cocoon (see "Remarks" under *Radoszkowskiana rufiventris*, below), but on cleared specimens, these lines clearly visible, each as fine line extending from dorsal midline toward spiracle on abdominal segments 1–7 (diagramed on fig. 49 as dotted line on third abdominal segment); ventral intersegmental lines moderately to weakly incised; paired dorsal body tubercles absent; middorsal body tubercles (see description in "Remarks" under *Radoszkowskiana rufiventris*) present or apparently absent; pleural swellings protuberant or not; abdominal segment 10 small, rounded in lateral profile (not pointed as in predefecating larva), attached in approximate middle of segment 9 in lateral view (figs. 49–52); anus positioned toward top of segment, with median crescentic, more or less varicose, somewhat raised area immediately dorsad of anus. Spiracles (figs. 49–52) moderately large, not or faintly pigmented, subequal in size; atrium globular, projecting various amounts above body wall, with indistinct rim; peritreme present, narrow to moderately wide; atrial inner surface with rows of denticles concentric with primary tracheal opening; primary tracheal opening with or apparently without collar.

Remarks: Interestingly, all exemplars examined consistently displayed three sensilla on each antennal papilla despite the fact that most were cleptoparasites representing three species. Three to four sensilla per antennal papilla, the usual number for nonparasitic bees, may be the plesiomorphic number for bee larvae. Often, but obviously not invariably, cleptoparasitic larvae have an increase in the number of antennal sensilla (Michelette et al., 2000; Roig-Alsina and Rozen, 1994; Rozen, 1996; Rozen et al. 2006), perhaps an adaptation enabling the larva to find the host immature or to detect competing cleptoparasitic larvae. However, the apid cleptoparasite *Coelioxoides waltheriae* is unique among bee larvae in that it apparently lacks antennae and antennal sensilla altogether (Alves-dos-Santos et al., 2002).

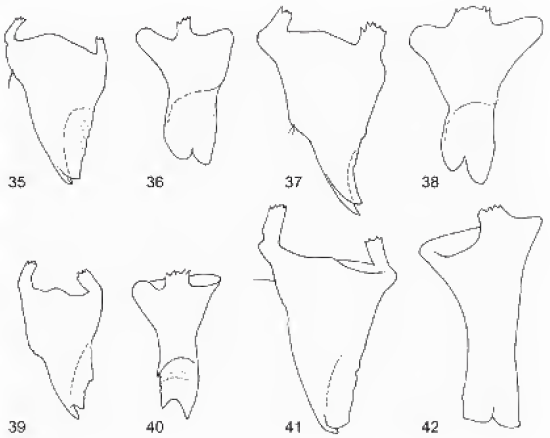
DESCRIPTIONS OF IMMATURES STAGES OF *RADOSZKOWSKIANA RUFIVENTRIS*

Egg/Mature Oocyte
figure 31

Diagnosis: The eggs of the four taxa dealt with here all appear to have a micropyle consisting of a cluster of polygons defined by raised edges. Elsewhere the chorion appears to



Figs. 32–34. Micropyles. **32.** On chorion covering first instar of *Radoszkowskiana rufiventris*. **33.** On oocytes of *Coelioxys coturnix*. **34.** On egg of *Megachile nigripes*.



Figs. 35–42. Right mandible of postdefecating larvae, dorsal and inner views, respectively, drawn to same scale. **35, 36.** *Radoszkowskiana rufiventris*. **37, 38.** *Coelioxys decipiens*. **39, 40.** *Coelioxys coturnix*. **41, 42.** *Megachile nigripes*. Note that apex of inner view of mandible of *R. rufiventris* is not in maximum profile as is the case in fig. 10.

be smooth and without features. See "Discussion of Egg Anatomy and Ovarian and Egg Statistics" and table 1 for ways of distinguishing species on the basis of egg size.

Description: See table 1 for dimensions and table 2 for egg index. Shape as seen in side view slightly curved, widest at middle, rear end tapering more than anterior end; as seen from above/below moderately elongate, parallel-sided, rounded at both ends, slightly more narrowly at rear in lateral view; micropyle not apparent under stereoscopic examination; under SEM examination, micropyle of egg (not oocyte) consisting of a cluster of polygons defined by raised borders (fig. 32). Color whitish; chorion under stereoscopic examination shiny, clear; under SEM examination remainder of chorion apparently without ornamentation.

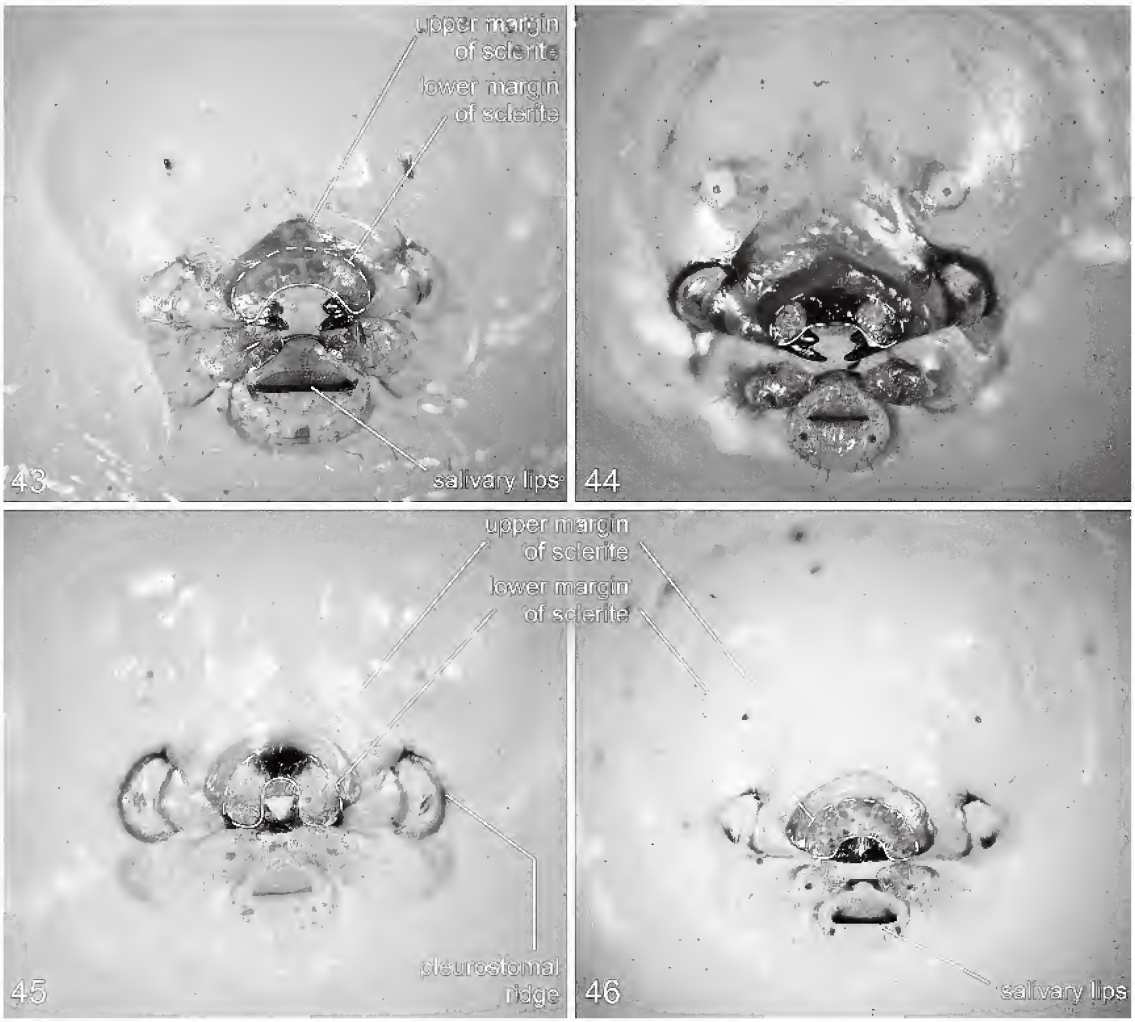
Material Examined: Seven oocytes, Egypt: Tel el Kebir, V-1–17, 2005 (J.G. Rozen, S.M. Kamel); 3 eggs, same except V-23–V1-1-2005.

Last Larval Instar

figures 9, 10, 35, 36, 43, 49, 53, 75–79

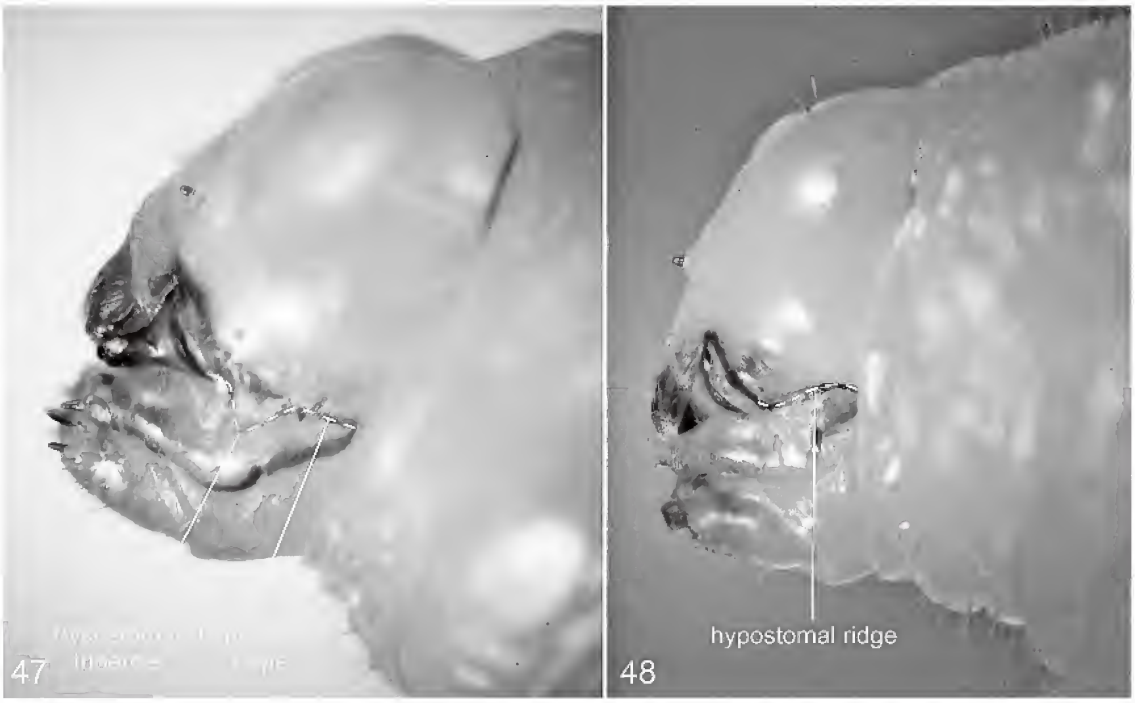
Diagnosis: The mature larvae *Radoszkowskiana rufiventris*, *Coelioxys decipiens*, and *Megachile nigripes* are similar in that they are robust, have comparatively small heads, and display considerable variation resulting from whether they have been constrained by cocoons or accidentally misshapen during preservation. Thus, lateral views of whole larvae (figs. 49–52) better illustrate their overall similarity rather than providing features of diagnostic value.

The mature larva of *Radoszkowskiana rufiventris* can be distinguished from that of



Figs. 43–46. Photographs of heads of postdefecating larvae, frontal views, with lower margin of labra defined by solid white line and with lower margin of labral sclerites defined by dashed line. **43.** *Radoszkowskiana rufiventris*. **44.** *Coelioxys decipiens*. **45.** *Coelioxys coturnix*. **46.** *Megachile nigripes*.

the host *Megachile* (*Pseudomegachile*) *nigripes* because (1) the upper tooth of the bidentate mandible is narrowly rounded apically (fig. 36) whereas that of *M. nigripes* is broadly subtruncate (fig. 42); (2) on uncleared specimens, the median part of the labral sclerite is darkly pigmented, widest medially, and tapers laterally, so that the dorsal margin of the sclerite is angled medially as seen in frontal view (fig. 43) compared with that of *M. nigripes*, which is less pigmented, more uniform in width across the labrum, and has its upper margin evenly curved along its entire length (fig. 46); (3) the labral apex and salivary lips are very broad in frontal view (fig. 43) compared with those of *M. nigripes* (fig. 46), so that in *R. rufiventris* the lips are much broader than one-half the distance between outer lateral margins of labral sclerite in frontal view, whereas the lips of *M. nigripes* are scarcely if at all as wide as one-half the distance between outer lateral margins of the labral sclerite; (4) the antennal papilla in lateral view (fig. 75) tapers gradually from base to apex, with apex broad whereas that of *M. nigripes* is more or less uniformly wide basally and tapers suddenly at about midlength to be narrowly rounded (fig. 93); and (5) the atrium



Figs. 47, 48. Photographs of heads of postdefecating larvae, lateral view. **47.** *Coelioxys decipiens*, with hypostomal tubercle and ridge delineated by dashed line. **48.** *Coelioxys coturnix*, with hypostomal ridge delineated by dashed line.

tends to be larger in diameter and the entire surface of the atrial wall bears circles of fine, long denticles concentric around the primary tracheal opening, whereas the atrium of *M. nigripes* is smaller in diameter and the atrial wall has shorter denticles restricted to one or two rings mostly immediately beneath the peritreme (and therefore easily overlooked) but not on the rest of the surface.

The last larval instar of *Radoszkowskiana rufiventris* can be easily separated from that of *Coelioxys decipiens* because the latter species possesses a pair of hypostomal tubercles (fig. 47), bearing an apical cluster of three to five long setae. Although varying in extent of expression, such tubercles are apparently characteristic for many species of *Coelioxys* (but not of *C. coturnix*, fig. 45, see below) and are unknown elsewhere among bee larvae. Unlike the postdefecating larvae of *R. rufiventris*, *M. nigripes*, and *M. minutissma*, those of *C. decipiens* and *C. coturnix* can easily be recognized because of a large darkly pigmented median spot, probably associated with sclerotization, extending from the labral sclerite to the lower labral margin. This spot, however, was absent on the early fifth-stage larval instar described below. The pleurostomal ridges of these two *Coelioxys* are also more deeply pigmented than those of the others treated here.

The much larger size of the mature larvae of *Radoszkowskiana rufiventris, Coelioxys decipiens*, and *Megachile nigripes* immediately distinguishes them from larvae of *C. coturnix* and its host, *M. minutissima*, both of which, unlike the other three species, are found in leaf-lined cells.

DESCRIPTION: Length (if straight) about 13 mm.

Head (figs. 43): Labral sclerite darkly pigmented (fig. 43); entire labral apex beyond sclerite pigmented (fig. 43) but not sclerotized, so that lower margin of sclerite obscured unless head capsule cleared, but labral apex without median area more darkly pigmented than lateral apical areas as in *Coelioxys* (figs. 44, 45); mandibular apices and basal thickening darkly pigmented; pleurostomal ridge pigmented only near mandibular articu-

lations, pigmentation fading between these points; hypostomal ridge, cardo, and base of stipes faintly pigmented; salivary lips, all palpi, apical part of stipes, antennal papilla, and premental sclerite pigmented. Hypostomal area normal, without downward-projecting, sensilla-bearing, hypostomal tubercle (called the pleurostomal thickening by Baker, 1971), such as found in *Coelioxys*. Antennal papilla gradually, evenly tapering from base to rounded apex, more than twice as long as basal diameter, with approximately three sensilla. Labral sclerite well developed, conspicuous because of pigmentation, its median part wide and its lateral arms tapering as seen in frontal view (fig. 43); labrum without large, darkly pigmented, median spot extending from labral sclerite to labral apex.

Mandible (figs. 35, 36) with dorsal tooth apically acutely rounded (best seen in fig. 10 where it is in maximum apical profile), shorter than ventral one; ventral tooth apically pointed; inner apical surface concave, forming apical concavity that is not sharply defined; cusp not developed; outer surface without tubercle, but with single conspicuous seta near base. Labiomaxillary region moderately strongly projecting in lateral view. Maxillary palpus moderately long, approximately equal in length to antennal papilla. Apex of labium exceedingly broad; labial palpus very long, obviously longer than maxillary palpus. Salivary lips exceedingly broad (fig. 43); microstructures within lips as shown in figures 76–79.

Body (fig. 49): Setae not as abundant as those of *Megachile nigripes* so that pleural area (swelling) of abdominal segment 8 with only about 8–10 setae. Pleural swellings not protuberant. Spiracles unpigmented, subequal in size except more posterior ones slightly smaller than those farther forward; atrium scarcely projecting above body wall, with indistinct rim; peritreme narrow, so that atrial opening large; atrial inner surface with rows of denticles concentric with primary tracheal opening; primary tracheal opening apparently without collar; subatrium narrow with outside width less than half maximum outside width of atrium; subatrium variable in length, with 6–12 chambers, with those at anterior end of body tending to be longer than those toward posterior end. Integumental sex characters unknown.

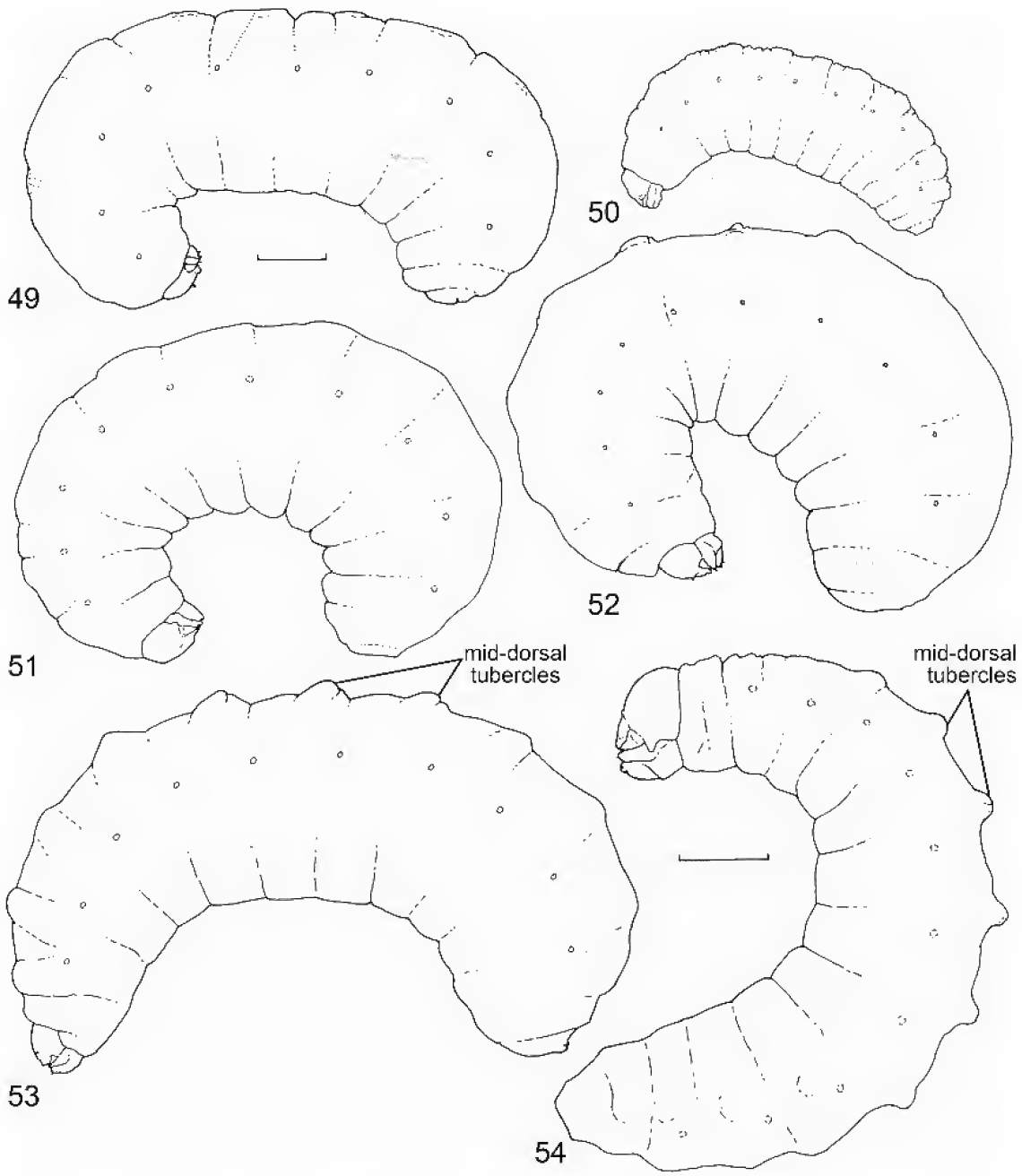
Predefecating form (fig. 53): As in postdefecating form except for following: integument smooth, not wrinkled. Dorsal intersegmental lines scarcely incised; ventral intersegmental line moderately incised; intrasegmental lines weak; abdominal segments 1–4 with caudal annulets more or less elevated medially forming middorsal tubercles; middorsal tubercles of segments 2–4 the most elevated, with their apices perhaps eversible; pleural swellings not developed; abdominal segment 10 small relative to preceding segment, apically pointed in lateral view, with dorsal lip pronounced; anus dorsal in position.

MATERIAL STUDIED: Five predefecating larvae (not all mature), Egypt: Tel el Kebir, collected V-19-2004, preserved V-20, 23-2004 (J.G. Rozen, S.M. Kamel); 5 postdefecating larvae, same except II-12-2005 (S.M. Kamel).

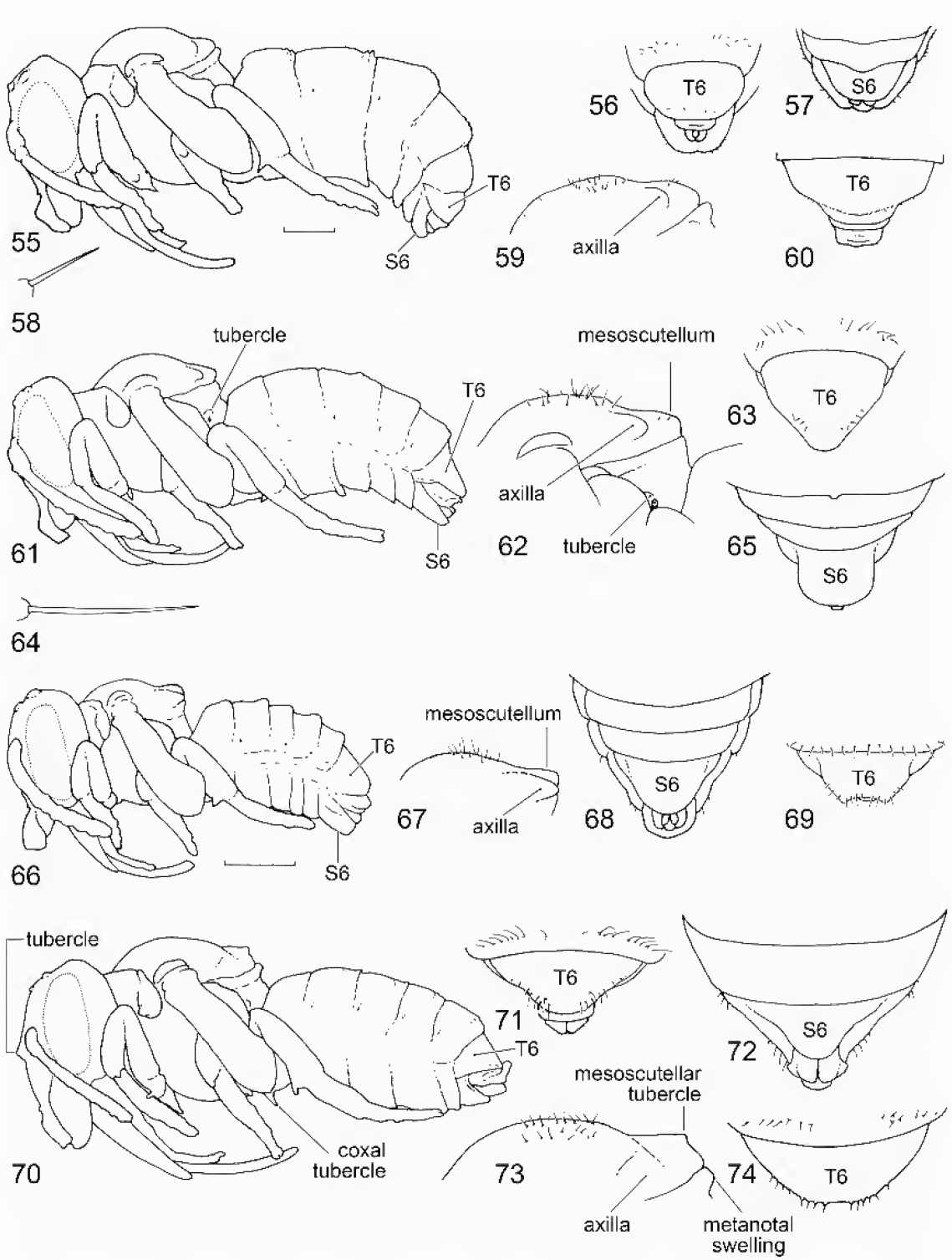
REMARKS: Pigmentation of the head capsule and appendages of the last instar develops over time, so that the pigmentation description, reported above, is found on the mature larva, both predefecating and postdefecating, but not on freshly emerged last larval instars. As has been noted elsewhere (Rozen and Özbek, 2004), clearing larval bees by boiling them in an aqueous solution of sodium hydroxide can modify pigmentation patterns in unexpected ways. In the case of *Radoszkowskiana rufiventris*, the dark pigmentation of the median section of the labral sclerite of a predefecating mature (or nearly mature) last instar was greatly reduced by such treatment, rendering nearly useless one of the important diagnostic features whereby the species could be distinguished from its host.

Although middorsal tubercles appear to be intersegmental, each presumably is on the midline of the posterior margin of the caudal annulet of the abdominal segment to which it is assigned. Found in larvae of many megachilids, they seem to be eversible and may be involved with locomotion. They are best developed on abdominal segments 1–4 and are most visible in larvae that are less than fully fed.

The middorsal swelling on the caudal annulets as well as the demarcations between cephalic and caudal annulets of the predefecating larva (fig. 53) become obscure on the



Figs. 49–52. Postdefecating larvae, lateral view; setae not depicted. **49.** *Radoszkowskiana rufiventris*; dotted line on dorsum of third abdominal segment indicates position of intrasegmental line. **50.** *Coelioxys coturnix*. **51.** *Coelioxys decipiens*. **52.** *Megachile nigripes*. Fig. 53. Predefecating larva of *R. rufiventris*. Fig. 54. Early last instar of *C. decipiens*. Scales (= 1.0 mm) refer to figs. 49–53 and fig. 54, respectively.



Figs. 55–60. Pupa of *Radoszkowskiana rufiventris*. **55.** Female, lateral view (setae omitted). **56.** Metasomal apex, dorsal view. **57.** Same, ventral view. **58.** Mesoscutal seta. **59.** Close-up of dorsum of mesonotum, lateral view. **60.** Male, metasomal apex, dorsal view. Figs. 61–65. Pupa of *Coelioxys*

postdefecating larva (fig. 49); these features are only variably discernable on some body segments of some of the postdefecating specimens. Obscuring of these elevations is due to compression of the very soft body integument of the postdefecating larva.

Pupa
figures 55–60

Diagnosis: The cleptoparasitic pupae treated here will correctly key to family (Rozen, 2000a) and can be distinguished from one another as follows: The pupa of *Radoszkowskiana rufiventris* can be separated from that of *Megachile nigripes* because the former lacks setae on the frons and pointed tubercles on the mid- and hindcoxae, and its midtrochanter is apically rounded, without an acute tubercle. Female pupae of *R. rufiventris* and *Coelioxys decipiens* differ from those of *M. nigripes* in that both species have a strongly projecting, broadly rounded to subtruncate S6 when viewed ventrally (figs. 57, 65) whereas the S6 of *M. nigripes* is short and tapers to a rounded point in ventral view (fig. 72). The pupa of *Coelioxys decipiens* can be distinguished from those of both *R. rufiventris* and *M. nigripes* because it possesses strongly projecting axillary spines (fig. 62) whereas the axillae of the other two species (fig. 59, 73) are scarcely produced and broadly rounded. The elongate female T6 and S6 (figs. 61, 63, 65) of *C. decipiens* also contrasts with the shorter ones of females of the other two species (figs. 56, 57, 71, 74). The pupa of *C. coturnix* shares the strongly projecting axillary spines characteristic of *C. decipiens*, but those of *C. coturnix* (fig. 67) extend farther backward than do those of *C. decipiens* (fig. 62). Females of these two species also posses an elongate T6, distinctly longer than the preceding tergum (figs. 61, 66). The much smaller size of the former immediately distinguishes these two congeners, as does the shape of the female S6s (figs. 65, 68). The pupa of *Megachile minutissima*, the host of *C. coturnix*, appears to have pointed axillae, particularly when the pigmented pupa is viewed from above, but these tubercles accommodate developing hairs and are far less pronounced than are the axillae of *C. coturnix*.

With all species treated here, the longer setae on dorsal mesosomal and metasomal surfaces arise from small tubercles. The lengths of the long setae vary from species to species, with those of *Coelioxys* (figs. 62, 64, 67) tending to be longer and more conspicuous than those of either *Radoszkowskiana rufiventris* (figs. 58, 59) or *Megachile nigripes* (fig. 73).

Head: Vertex with band of scattered short setae that do not extend below level of median ocellus; supraclypeal area lacking median tubercle, other tubercles and verrucae lacking. Discal area of labrum strongly protuberant as seen in lateral view (fig. 55); pupal ocelli moderately defined, without strong tubercles. Mandible simple, without subapical ventral swelling for developing adult setae.

Mesosoma: Mesoscutum with scattered, inconspicuous, short setae, which extend onto mesoscutellum. Lateral angle of pronotum finely pointed, not tuberculate; lateral lobe of pronotum moderately projecting, apically rounded. Mesoscutum without tubercles or verrucae; mesoscutellum without median, low, rounded projection; axillae obtusely rounded, not strongly projecting backward; metanotum with slight median swelling; propodeum on each side without tubercle near spiracle; mesepisternum without tubercles. Tegula not produced, without tubercles or verrucae; wings without tubercles. Forecoxa with acute

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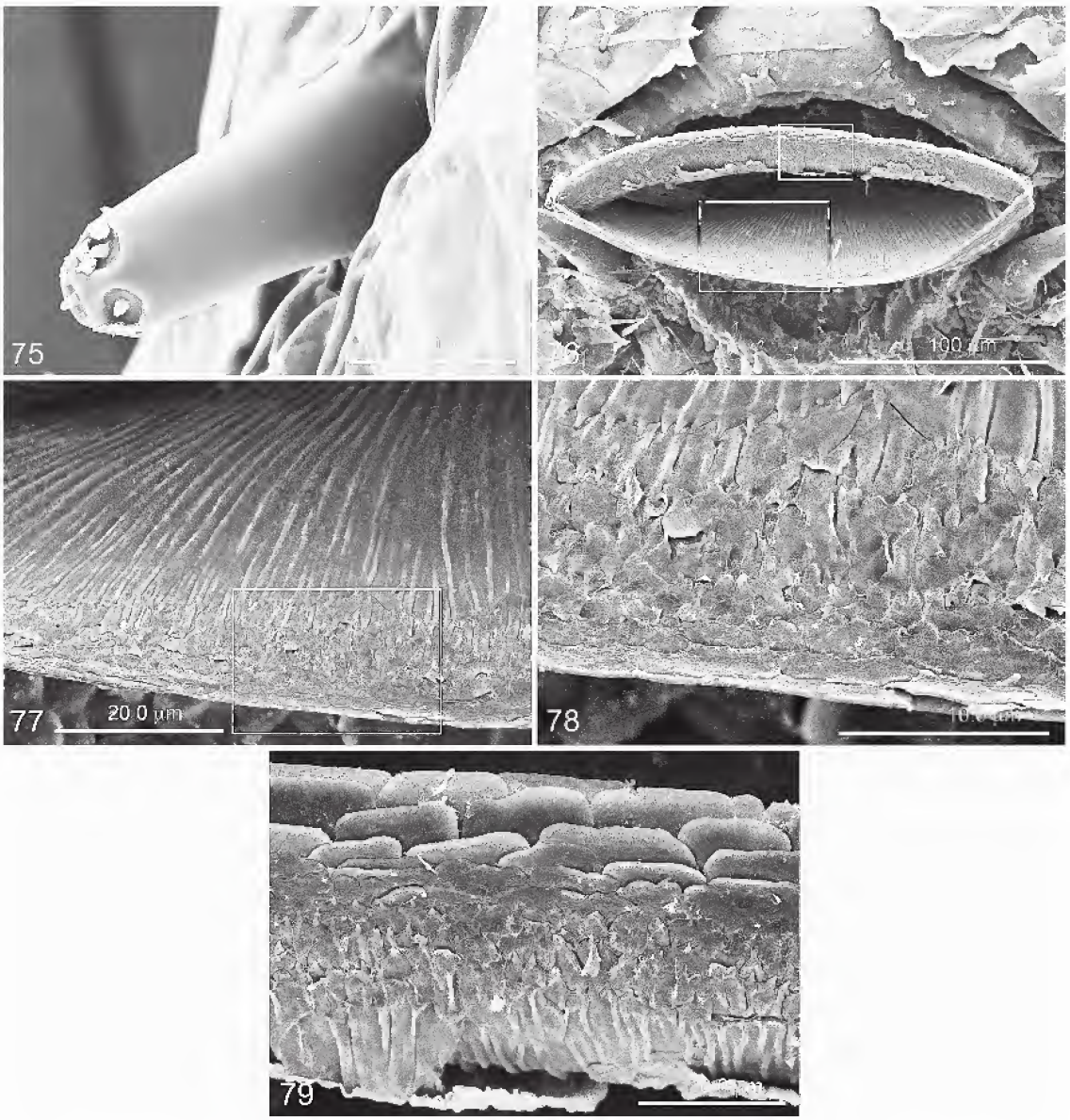
decipients. **61.** Female, lateral view (setae omitted). **62.** Close-up of dorsum of mesonotum, metanotum, and propodeum, lateral view. **63.** Metasomal apex, dorsal view. **64.** Mesoscutal seta. **65.** Metasomal apex, ventral view. Figs. 66–69. Pupa of *Coelioxys coturnix*. **66.** Female, lateral view (setae omitted). **67.** Close-up of dorsum of mesonotum, lateral view. **68.** Metasomal apex, ventral view. **69.** Male, metasomal apex, dorsal view. Figs. 70–74. Pupa of *Megachile nigripes*. **70.** Female, lateral view (setae omitted). **71.** Metasomal apex, dorsal view. **72.** Same, ventral view. **73.** Close-up of dorsum of mesonotum, lateral view. **74.** Male, apex of metasoma, dorsal view. Scale lines (= 1.0 mm) refer to figs. 55, 61, 70 and to 66, respectively. Figures 58, 64 to same scale.



Figs. 75–79. SEM micrographs of the postdefecating larva of *Radoszkowskiana rufiventris*. **75.** Antennal papilla, lateral view. **76.** Salivary lips, frontal view. **77.** Close-up of inner surface of lower lip identified by rectangle in fig. 76. **78.** Close-up of inner surface of lower lip identified by rectangle in fig. 77. **79.** Close-up of inner surface of upper lip identified in fig. 77.

apical tubercle; mid- and hindcoxae apically rounded; foretrochanter with acute ventroapical tuberclelike projection; mid- and hindtrochanters ventroapically produced but apex rounded; all femora unmodified, without tubercles; fore- and midtibiae each with small, apical tubercle on outer surface; hindtibia apically produced as rounded tubercle; all tarsi unremarkable.

METASOMA: Terga with apical bands of inconspicuous, moderately short setae, some rising from minute tubercles, distributed as follows: T1 with band reduced to few setae found sublaterally; T2 –T5 with bands more

complete than on T1, but with most setae found sublaterally, not medially; T6 with a few setae apically. T6 of female much wider than long, sides curving outward, apex broad, transverse, nearly straight (fig. 56); T6 of male much wider than long, with curved posterior margin (fig. 60). Sterna without conspicuous setae, although S6 with very fine, scattered setae; S6 of female projecting posteriorly, extending beyond anal area (but not elongate as in *Coelioxys decipiens*, fig. 65), apically broad, indistinctly subtruncate, faintly bilobed. Apex of metasoma apparently without terminal spine.

Material Studied: Two female and 3 male pupae, Egypt: Tel el Kebir, IV-1–7-2005 (S.M. Kamel).

DESCRIPTIONS OF IMMATURE STAGES OF *COELIOXYS (LIOTHYRAPIS) DECIPIENS*

Mature Oocyte
figure 31

Diagnosis: See "Discussion of Egg Anatomy and Ovarian and Egg Statistics" and table 1 for ways of recognizing this species on the basis of egg size.

Description: See table 1 for dimensions and table 2 for egg index. Shape as seen in side view faintly curved, sometimes venter straight rather than slightly concave although dorsum curved in outline; oocyte widest around middle, anterior end usually tapering less than posterior end but sometimes indistinguishable; as seen from above/below moderately elongate, parallel-sided, rounded at both ends; micropyle not observed. Color whitish; chorion under stereoscopic examination shiny, clear; not examined under SEM.

Material Examined: Six mature oocytes, Egypt: Tel el Kebir, V-23, 29-2005 (J.G. Rozen, S.M. Kamel).

Remarks: Eggs of this species were not available for examination. The mature oocytes when studied with the SEM appeared to have a single small pore at their anterior end as did the mature oocytes of *Radoszkowskiana rufiventris*. We think that the cluster of chorionic polygons had not yet been deposited, as was also the case with oocytes of *R. rufiventris*.

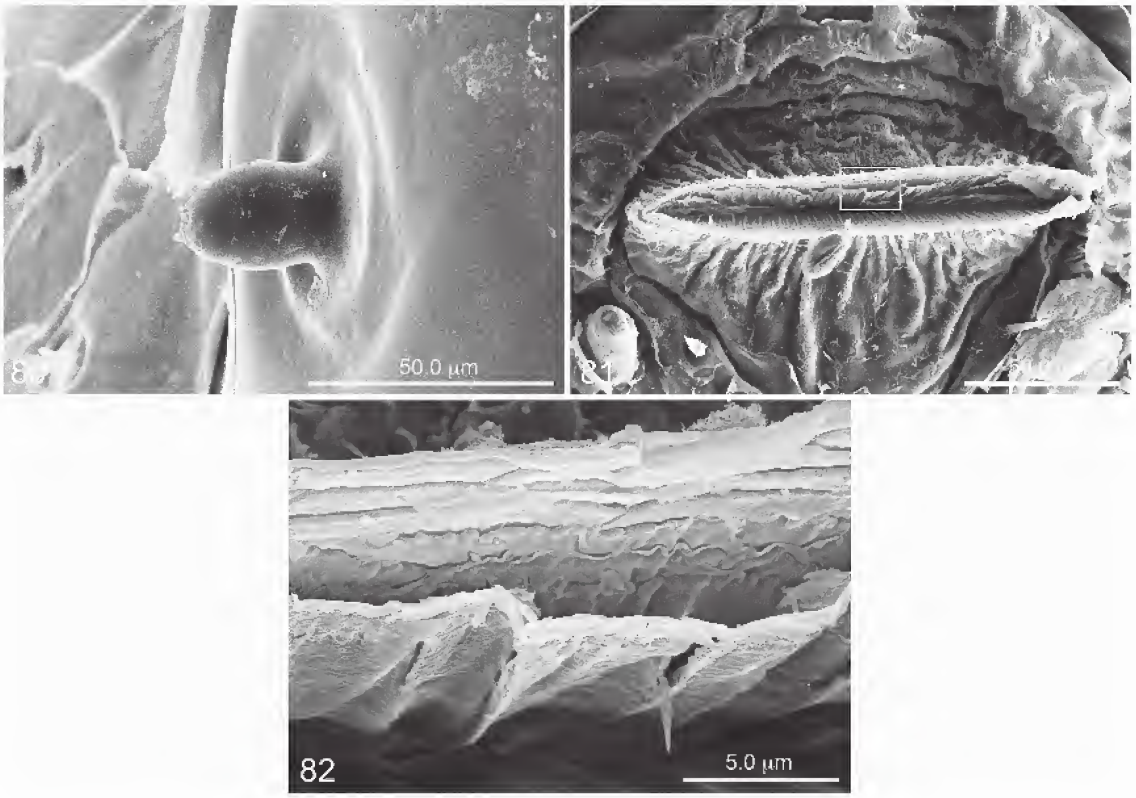
Last Larval Instar
figures 37, 38, 44, 47, 51, 54, 80–82

In addition to the postdefecating larvae described below, an early fifth instar was collected and is treated following the description of the postdefecating forms. Its pronounced middorsal swellings are typical of such swelling on similar early-stage larvae of the other species.

Diagnosis: Please see the diagnosis of the last larval instar of *Radoszkowskiana rufiventris* (above) for ways of distinguishing the mature larva of *Coelioxys decipiens* from those of *Radoszkowskiana rufiventris* and *Megachile nigripes*. Because the mature larva of *C. coturnix* is found in a leaf-lined host cell and is much smaller than that of *C. decipiens*, they are unlikely to be confused. The most obvious anatomical difference between these two species is that *C. coturnix* lacks hypostomal tubercles, which are a generic identifying feature of most other members of the genus.

Description: Length (if straight) about 11 mm.

Head (figs. 44, 47): Pigmentation as described for *Radoszkowskiana rufiventris* except for following: labral apex with large, very dark median area, darker than pigmentation of labral sclerite (fig. 44); pleurostomal ridge darkly pigmented its entire length (fig. 44); hypostomal ridge, cardo, and base of stipes darkly pigmented. Area immediately above hypostomal ridge posterior to posterior mandibular articulation produced as downward swelling, here termed hypostomal tubercle (fig. 47) (called the pleurostomal thickening by Baker [1971] and genal projection by Rozen [2001]); this tubercle bearing apically approximately two to five downward-pointing, long sensilla and on its lateral surface a scattering of nonsetiform sensilla. Antenna papilla as seen with SEM apically slightly swollen, about twice as long as maximum diameter, with approximately three sensilla. Labral sclerite weakly pigmented, arched medially, its median part wide, somewhat wider than lateral arms as seen in frontal view (fig. 44); labrum with large, darkly pigmented, nonsclerotized, median spot extending from paler labral sclerite to apical margin of labrum (fig. 44).



Figs. 80–82. SEM micrographs of the postdefecating larva of *Coelioxys decipiens*. **80.** Antennal papilla, lateral view. **81.** Salivary lips, frontal view. **82.** Close-up of inner surface of upper lip identified by rectangle in fig. 81.

Mandible (figs. 37, 38) with dorsal tooth apically rounded to weakly subtruncate, shorter than ventral one; ventral tooth narrowly rounded to subtruncate apically; inner apical surface forming shallow apical concavity; outer surface with two faint tubercles, each with conspicuous seta. Maxillary palpus moderately long, length more than twice as long as basal diameter. Apex of labium normally narrow (fig. 44); maxillary and labial palpi long, subequal in length. Salivary lips as broad as, to slightly less broad than, distance between bases of labial palpi, which are normally spaced on normally narrow labial apex; microstructures within lips as shown in figures 81 and 82.

Body (fig. 51): Setae not as abundant as those of *Megachile nigripes* so that pleural area (swelling) of abdominal segment 8 with only about five to six setae. Pleural swellings more or less evident on abdominal segments 1–8 (well developed on some earlier larval stages). Anus positioned toward top of segment, with median crescentic, more or less varicose, somewhat raised area immediately dorsad of anus. Spiracles unpigmented, subequal in size; atrium projecting well above body wall, with rim; peritreme moderately wide; atrial inner surface with rows of denticles concentric with primary tracheal opening; primary tracheal opening with collar; subatrium moderately narrow with outside width about half maximum outside width of atrium; subatrium moderate in length, with about 10–12 chambers. Male with median, transverse, short integumental scar ventrally near posterior margin of abdominal segment 9; female integumental sex characters unknown.

Early last larval instar (fig. 54): As in postdefecating form except for following: mandible with both teeth apically pointed. Labrum without large dark median spot as

found on postdefecating larvae. Integument smooth, not wrinkled. Dorsal intersegmental lines scarcely incised; ventral intersegmental line moderately incised; intrasegmental lines absent; metathorax with low posterior middorsal tubercle; abdominal segments 1–5 with caudal annulets more or less elevated medially forming pronounced middorsal tubercles, their apices perhaps eversible; pleural swellings of abdominal segments 1–9 well developed; venter of abdominal segment 9 apparently slightly produced; anatomy of abdominal segment 10 uncertain because of possible postmortem preservation changes.

Material Examined: Three postdefecating larvae, Egypt: Tel el Kebir, II-12-2005 (S.M. Kamel); numerous postdefecating larvae, same except XII-20-2005; 1 early fifth instar, same except V-27-2005 (J.G. Rozen, S.M. Kamel).

Remarks: The more rounded mandibular apices of postdefecating larvae contrasting with the more pointed mandibular apices of the early-stage larvae probably results from wear.

The middorsal tubercles of the metathorax and first five abdominal segments appear to be intersegmental on the early stage fifth instar, but the intersegmental lines actually traverse the dorsum immediately behind them on the cleared specimen.

Pupa
figures 61–65

Diagnosis: Please see diagnosis of pupal *Radoszkowskiana rufiventris*.

Head: As described for *Radoszkowskiana rufiventris* except for following: setae on vertex tending to be longer, more conspicuous, mostly restricted to area above level of median ocellus but a few very short setae scattered below median ocellus.

Mesosoma: As described for *Radoszkowskiana rufiventris* except for following: mesoscutal (but not mesoscutellar) setae, longer, more conspicuous; mesoscutellum with only one or two setae. Mesoscutellum with faint median posterior rounded tubercle; axillae projecting backward with apex acute although rounded, well separated from mesoscutellum; apices of axillae not extending posteriorly as far as posterior median tubercle of mesoscutellum; metanotum without median swelling; propodeum on each side with low but distinct tubercle near spiracle (fig. 61). Midcoxa with small ventroapical tubercle; all trochanters without acute ventroapical projection; midtibia with small, apical swelling on outer surface too rounded to be termed tubercle.

Metasoma: As described for *Radoszkowskiana rufiventris* except for following: tergal setae longer, more conspicuous than those of *R. rufiventris*; those of T1 absent (or nearly so); T2–T5 with seta bands well developed, tending not to be interrupted medially. T6 of female elongate, in dorsal view about as wide basally as length, sides concave in apical half in dorsal view, apex narrowly rounded (fig. 63). S6 elongate, almost parallel-sided (fig. 65).

Material Studied: Two female pupae (male unknown), Egypt: Tel el Kebir, IV-1–7-2005 (S.M. Kamel).

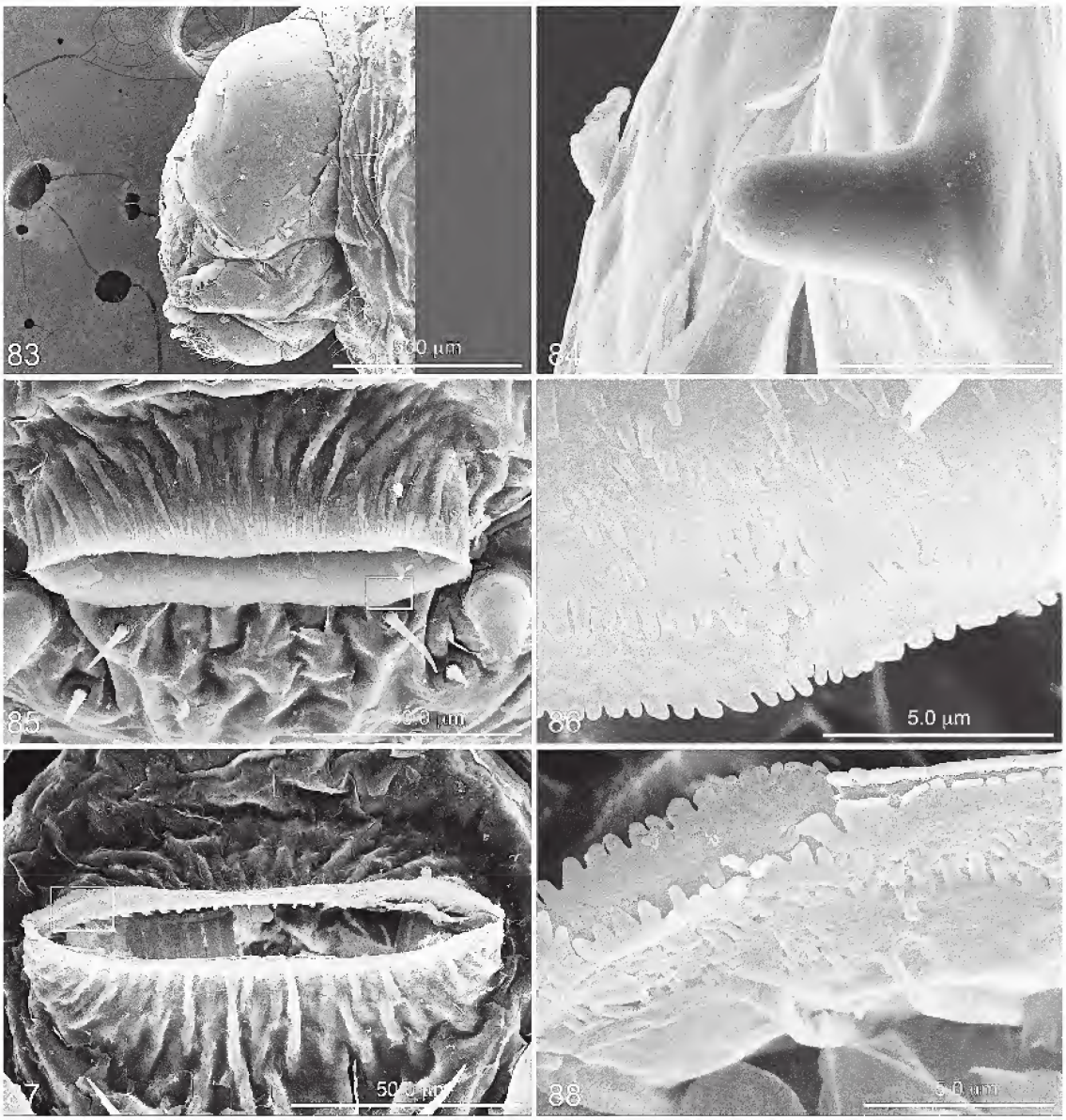
DESCRIPTION OF THE IMMATURE STAGES OF *COELIOXYS* (*ALLOCOELIOXYS*) *COTURNIX*

Mature Oocyte
figures 31, 33

Diagnosis: See "Discussion of Egg Anatomy and Ovarian and Egg Statistics" and table 1 for ways of recognizing this species on the basis of egg size.

Description: See table 1 for dimensions and table 2 for egg index. Shape as seen in side view slightly curved, widest in anterior half to posterior half, toward middle; posterior half tapering to more narrowly rounded posterior end than anterior end; as seen from above/below moderately elongate, parallel-sided, rounded at both ends; micropyle a cluster of polygons with raised edges at anterior pole (fig. 33). Color whitish; chorion under stereoscopic examination shiny, transparent; under SEM examination apparently without chorionic features other than micropylar area.

Material Examined: Two mature oocytes, Egypt: Tel el Kebir, V-22-2005 (J.G. Rozen); 2 mature oocytes, same except V-24-2005 (M. Shebl).

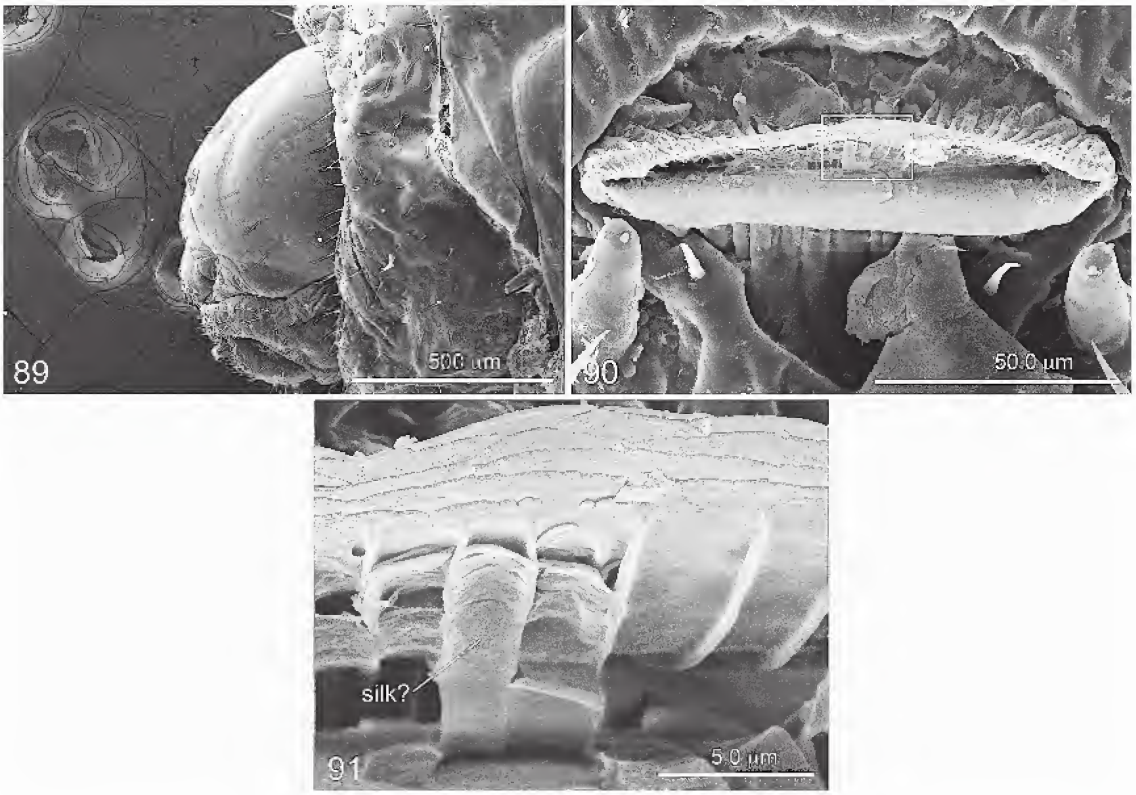


Figs. 83–88. SEM micrographs of the postdefecating larva of *Coelioxys coturnix*. **83.** Head, lateral view, showing strongly curved profile of cranium. **84.** Antennal papilla, lateral view. **85.** Salivary lips from above. **86.** Close-up of inner surface of lower lip identified by rectangle in fig. 85. **87.** Salivary lips, frontal view. **88.** Close-up of inner surface of upper lip, identified by rectangle in fig. 87.

Last Larval Instar
figures 39, 40 45, 48, 50, 83–88

Diagnosis: See diagnoses of the mature larvae of *Radoszkowskiana rufiventris* and *Coelioxys decipiens* (above). Although there are no shared structural features of fifth instars of *C. coturnix* and *C. decipiens*, pronounced similarities in pigmentation in the head capsule easily distinguishes them from *R. rufiventris*, *Megachile minutissima*, and *M. nigripes*.

The postdefecating larva of *Coelioxys coturnix* can be distinguished from that of its host, *Megachile minutissima*, because, unlike in the latter species, the pleurostomal and



Figs. 89–91. SEM micrographs of the postdefecating larva of *Megachile minutissima*. **89.** Head, lateral view, showing slopping profile of cranium. **90.** Salivary lips, frontal view. **91.** Close-up of upper lip identified by rectangle in fig. 90.

hypostomal ridges are darkly pigmented (figs. 45, 48). Furthermore, whereas the upper part of the head capsule of *M. minutissima* is evenly sloping in lateral view (fig. 89), that of *C. coturnix* is abruptly curved (fig. 83). The salivary lips of these two species differ in that those of *C. coturnix* are papillate (figs. 86, 88) and those of the host are not (fig. 91).

Description: Length if straight about 5.5 mm.

Head (figs. 45, 48): Pigmentation as described for *Coelioxys decipiens* except for following: labral apex (fig. 45) with darkly pigmented median area much narrower than that of *C. decipiens*; lateral apical areas unpigmented. Hypostomal area normal (fig. 48), without downward-projecting, sensilla-bearing, hypostomal tubercle as found in *C. decipiens*. Antennal papilla gradually tapering from base to rounded apex, somewhat longer than basal diameter, with approximately three sensilla. Labral sclerite weakly pigmented, scarcely wider medially than laterally (fig. 45); labrum with narrow, darkly pigmented, median spot apically (fig. 45); lower apical margin deeply emarginate, more so than in other species (figs. 45); apical lateral areas unpigmented.

Mandible (figs. 38, 40) with dorsal tooth acutely pointed, shorter than ventral one; ventral tooth acutely pointed; upper apical edge projecting and irregularly jagged, forming apical concavity; cusp not developed; outer surface without tubercle, but with swelling toward base that may bear sensilla. Labiomaxillary region moderately projecting in lateral view (fig. 48). Maxillary palpus moderately long, distinctly longer than antennal papilla. Apex of labium normally narrow; labial palpus moderately long, equal to or longer than maxillary palpus. Salivary lips approximately as broad as distance between

bases of labial palpi, which are normally separated on normally narrow labial apex; microstructures within lips as shown in figures 85–88.

Body (fig. 50): Pleural swellings slightly protuberant; pleural area (swelling) of abdominal segment 8 with only about three to four setae. Anus positioned toward top of segment, with median crescentic, somewhat raised area immediately dorsad of anus, this area not varicose. Spiracles unpigmented, subequal in size; atrium projecting above body wall, with rim; peritreme moderately narrow; atrial inner surface with rows of denticles concentric with primary tracheal opening; primary tracheal opening presumably with collar; subatrium moderately narrow, tapering from outside in; subatrium moderately short, with about eight chambers. Integumental sex characters of male a ventral, transverse, median scar toward posterior end of abdominal segment 9.

Predefecating form: Unknown.

Material Examined: Seven postdefecating larvae, Egypt: Suez Canal University, Ismailia, XII-27-2005 (S.M. Kamel); 5 postdefecating larvae, Egypt: Tel el Kebir, XII-20-2005 (S.M. Kamel).

Remarks: A faint lateral exterior swelling at the junction of the pleural and hypostomal ridges bears one or two setae that are about the same length of other cephalic setae. Because this swelling does not extend downward and is not positioned posterior to this junction, it is probably not a vestige of the hypostomal tubercle of such other *Coelioxys* as *C. decipiens*.

Pupa
figures 66–69

Diagnosis: Please see diagnosis of pupa of *Radoszkowskiana rufiventris*.

Head: As described for *Radoszkowskiana rufiventris* except for following: setae of vertex long relative to body size, as in *Coelioxys decipiens*.

Mesosoma: As described for *Radoszkowskiana rufiventris* except for following: mesoscutal setae long compared with body size, moderately conspicuous; scutellum without setae. Axillae strongly projecting backward so that apices in line with posterior edge of mesoscutellum (fig. 67), thus more pronounced than those of *Coelioxys decipiens*; metanotum with slight, median, posterior swelling. Leg segments without distinct projections or tubercles.

Metasoma: As described for *Radoszkowskiana rufiventris* except for following: tergal setae moderately long and conspicuous. T1 without setae or with just one or two on each side; T2–T5 each with setae evenly distributed in posterior bands. T6 of female somewhat wider than long, sides vaguely concave, apex rounded; T6 of male apically bilobed (fig. 69). S6 of female with sides gradually tapering to rounded apex in ventral view (fig. 68). Apex of metasoma apparently without terminal spine.

Material Studied: Two female and 1 male pupae, Egypt: Suez Canal University, Ismailia, XII-27-2005 (S.M. Kamel).

DESCRIPTIONS OF IMMATURE STAGES OF *MEGACHILE* (*PSEUDOMEGACHILE*) *NIGRIPES*

Egg/Mature Oocyte
figures 31, 34

Diagnosis: See "Discussion of Egg Anatomy and Ovarian and Egg Statistics" and table 1 for ways of recognizing this species on the basis of egg size.

Description: See table 1 for dimensions and table 2 for egg index. Shape as seen in side view slightly curved, widest around middle, anterior end tapering slightly more than posterior end; as seen from above/below moderately elongate, parallel-sided, rounded at both ends, slightly more narrowly so at front (possibly result of having been constricted by follicular tissue); micropyle not apparent under stereoscopic examination; under SEM micropyle consisting of a cluster of polygons with raised edges (fig. 34). Color whitish; chorion under stereoscopic examination shiny, clear; under SEM examination apparently without chorionic features except for micropylar area.

Material Examined: One mature oocyte, Egypt: Tel el Kebir, V-27-2005 (J.G. Rozen, S.M. Kamel); 1 egg, same except VI-1-2005 (M. Shebl); 1 egg, same except V1-18-2004

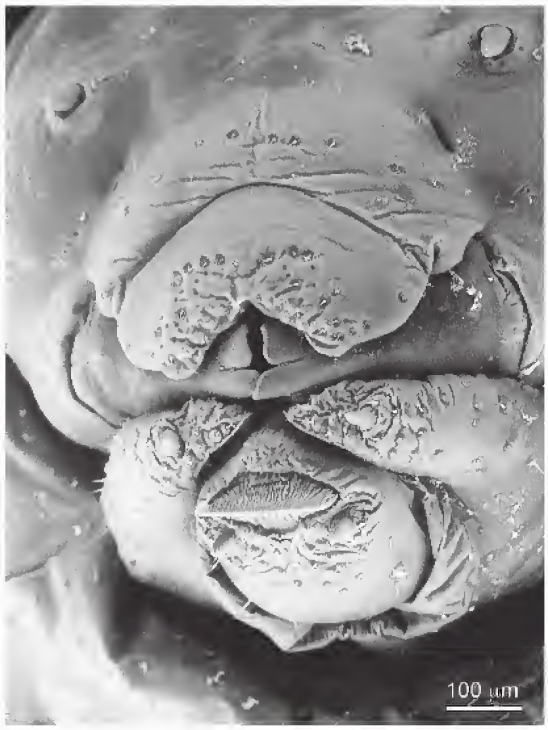


Fig. 92. Extended variable-pressure SEM micrograph of face of postdefecating larva of *Megachile nigripes*, oblique frontal view showing salivary lips normally closed in contrast to lips on specimen that has been subjected to critical point drying, for example, figs. 94–98.

(J.G. Rozen); 2 eggs same except V-22-2004 (S.M. Kamel).

Last Larval Instar
figures 41, 42, 46, 52, 92–98

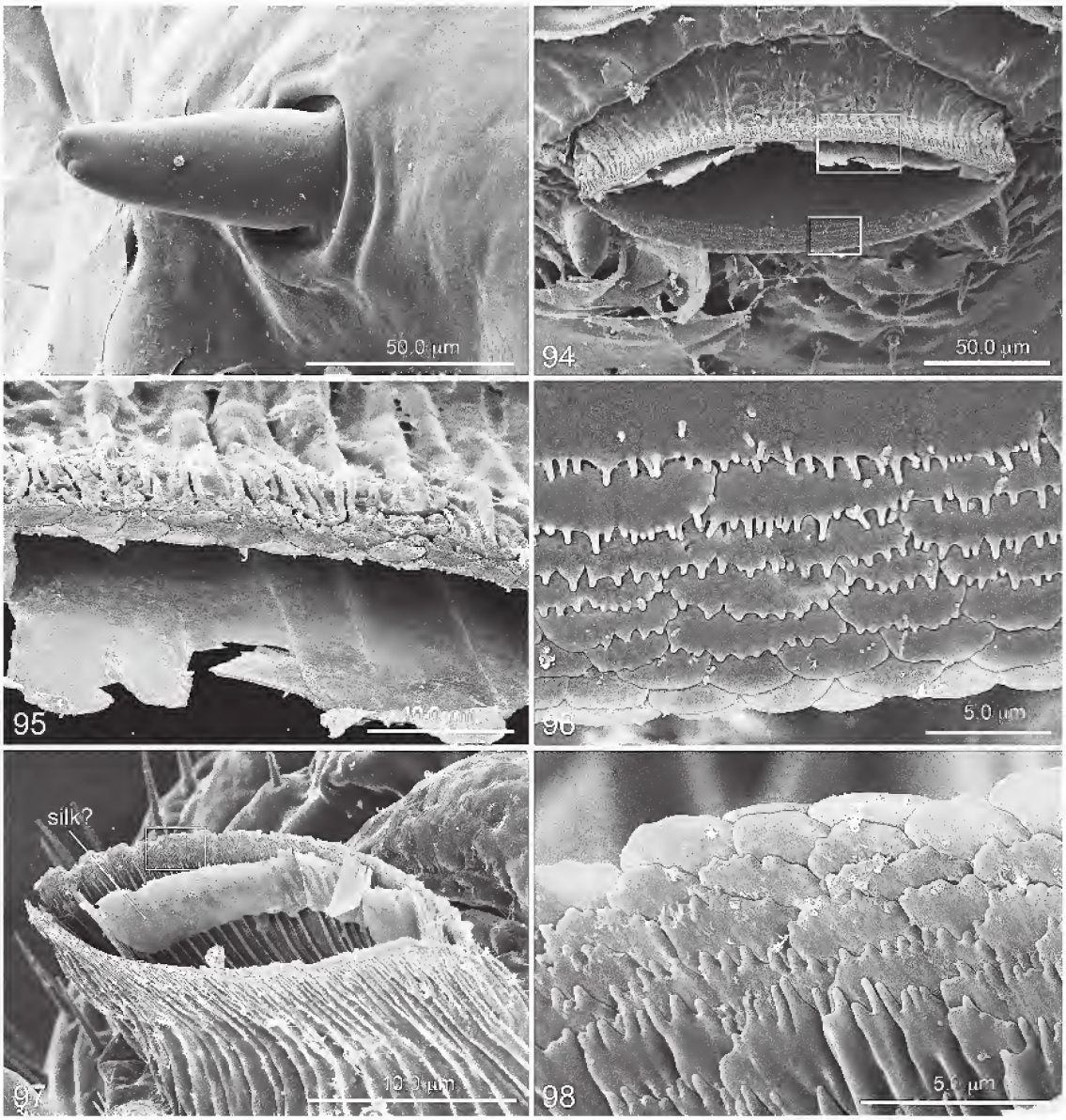
Diagnosis: The mature larva of *Megachile nigripes* can be distinguished from those of *Radoszkowskiana rufiventris* and *Coelioxys decipiens* by the features given in the diagnoses of the two cleptoparasites.

Description: Length (if straight) about 14 mm.

Head (fig. 46): Pigmentation as described for *Radoszkowskiana rufiventris* except for following: labral sclerite weakly pigmented (fig. 46); entire labral apex beyond sclerite pigmented but not sclerotized, so that lower margin of sclerite obscured unless head capsule cleared; labral apex without median area more darkly pigmented than lateral apical areas as in *Coelioxys decipiens*. Hypostomal area normal, without downward-projecting, sensilla-bearing tubercle, such as found in *Coelioxys*. Contrary to that of *Radoszkowskiana rufiventris*, antennal papilla with apical half tapering to narrowly rounded apex (fig. 93), about twice as long as basal diameter, with approximately three sensilla. Labral sclerite (fig. 46) moderately developed, less conspicuous that that of *R. rufiventris* because of lack of pigmentation, especially medially, its median part not substantially wider than lateral arms as seen in frontal view; labrum without large, median, darkly pigmented spot extending from labral sclerite to labral apex.

Mandible (figs. 41, 42) with dorsal tooth apically broadly subtruncate, approximately equal in length to ventral one; ventral tooth apically subtruncate, narrower than dorsal tooth; inner apical surface forming indistinct apical concavity; cusp not developed; outer surface with indistinct tubercle bearing conspicuous seta near base. Maxillary palpus moderately long, approximately equal in length to antennal papilla. Apex of labium (fig. 46) broad, but not exceedingly so, as in *R. rufiventris*; labial and maxillary palpi approximately equal in length. Salivary lips not quite as broad as distance between bases of labial palpi, which are themselves normally spaced; microstructures within lips as shown in figures 94–98.

Body (fig. 52): Setae somewhat shorter and far more dense than those of *Radoszkowskiana rufiventris* and *Coelioxys decipiens*, so the pleural swelling of abdominal segment 8 with approximately 40 setae. Midbody tubercles more or less evident on metathorax and abdominal segments 1–4, depending on specimen (sometimes obscured by compression); pleural swellings not protuberant. Spiracles faintly pigmented, subequal in size; atrium projecting above body wall, with distinct but fine rim; peritreme narrow; atrial inner surface with several short rows of denticles below peritreme, concentric with primary tracheal opening; primary tracheal opening with collar; subatrium with outside width approximately half maximum outside width of atrium; subatrium variable in length, with 8–12



Figs. 93–98. SEM micrographs of the postdefecating larva of *Megachile nigripes*. **93.** Antennal papillae, lateral view. **94.** Salivary lips, approximate frontal view. **95.** Close-up of upper lip identified in rectangle in fig. 94. **96.** Close-up of lower salivary lip identified by rectangle in fig. 94. **97.** Salivary lips from below. **98.** Close-up of upper lip identified by rectangle in fig. 97.

chambers. Integumental sex characters unknown.

Predefecating form: As described for postdefecating form except for following: integument smooth, not wrinkled. Abdominal segments 1–5 with caudal annulets more or less elevated medially, with those of segments 3–5 the most elevated, their apices perhaps eversible; pleural swellings not developed; abdominal segment 10 apically pointed in lateral view, with dorsal lip pronounced; anus dorsal in position.

Material Examined: Numerous predefecating larvae, Egypt: Tel el Kebir, 30°33′30″N, 31°56′13″E, collected V-19-2004, preserved V-20, 23-2004 (J.G. Rozen, S.M. Kamel);

numerous postdefecating larvae, same data, except II-12-2005 (S.M. Kamel).

Pupa
figures 70–74

Diagnosis: Please see diagnosis of pupal *Radoszkowskiana rufiventris* (above).

Head: As described for *Radoszkowskiana rufiventris* except for following: vertex with band of scattered moderately long setae; shorter setae present on frons and supraclypeal area. Supraclypeal area of female, but not male, with median tubercle (fig. 70).

Mesosoma: As described for *Radoszkowskiana rufiventris* except for following: mesoscutum with scattered moderately long setae; mesoscutellum without setae. Lateral angle of pronotum tuberculate; lateral lobe of pronotum pronounced, acutely rounded apically. Mesoscutellum with median, low, rounded tubercle; axillae broadly rounded, not projecting backward; metanotum with median swelling. Forecoxa with stout, posteriorly directed, apical tubercle, and male forecoxa also with downward-directed, blunt tubercle; mid- and hindcoxae each with pronounced apical tubercle; foretrochanter with pronounced, acute ventroapical tubercle; midtrochanter with acute ventroapical tubercle; hindtrochanter apically rounded in female but apically attenuated, tuberclelike in male; fore- and midtibiae each with small, apical tubercle on outer surface, this tubercle almost absent in male; hindtibia without apical tubercle.

Metasoma: As described for *Radoszkowskiana rufiventris* except for following: T1 without band of seta; T2–T6 each with posterior band of moderately long setae, most dense sublaterally. T6 much wider than long, posterior margin curved as seen dorsally (female, fig. 71) or faintly bilobed (male, fig. 74). S6 of female as seen in ventral view (fig. 72) short, tapering to narrowly round apex, exposing apices of gonostyli. Apex of metasoma with presumably eversible, median, apically blunt, terminal lobe evident immediately above anus on many specimens (fig. 70).

Material Studied: Numerous female and male pupae, Egypt: Tel el Kebir, IV-1–7-2005 (S.M. Kamel).

Remarks: The apical metasomal lobe appears to be a variable feature, pronounced on some specimens but less so on others. The variability of this feature suggests that it may be eversible. Although not clearly evident on the cleptoparasitic taxa treated herein, the apparent absence could be simply a sampling error resulting from the examination of too few pupae of the cleptoparasites.

DISCUSSION OF EGG ANATOMY AND OVARIAN AND EGG STATISTICS

Except for the matter of size, the eggs/mature oocytes of the four taxa dealt with here are apparently quite similar. Although there appear to be subtle differences in shape, variability within a taxon also exists, so that it would be difficult to differentiate taxa on the bases of the anatomy (exclusive of size) of their eggs. Extreme modifications of shape and chorionic ornamentation found among various cleptoparasitic apids (e.g., Rozen, 2003; Rozen and Özbek. 2003) do not exist among these taxa except in the case of most other species of *Coelioxys*, which have the extreme anterior part of the egg expanded laterally into a shape of a "horseshoe nail" (Graenicher, 1905; Iwata, 1939, 1965; Bohart, 1970; Baker, 1971; Rozen, 2003). The swelling at the anterior end may be associated with species that deposit their eggs in *Megachile* brood cells that are lined with leaves; the swelling presumably assists in attaching and perhaps hiding the eggs from returning host females. The absence of the modification in eggs of *C. decipiens* (also noted by Iwata, 1965), *C. coturnix*, and *Radoszkowskiana rufiventris* may possibly be explained if their eggs are inserted into the host cell at a time when the host female will no longer inspect the cell for parasite eggs, as seems to be the case at least with *R. rufiventris*.

Table 1 permits comparisons of the dimensions of the oocytes/eggs addressed in this study. The most notable statistic is the very large size of the mature oocyte/egg of *Megachile nigripes* compared with those of its two cleptoparasites (also reflected in the egg indices of the three species, table 2). Those of *Radoszkowskiana rufiventris* and *Coelioxys*

TABLE 1
Egg/Mature Oocyte Dimensions of Bees in Current Study
Numbers in the first three columns are means if more than one specimen was examined. Where a specimen contained more than one mature oocyte, only the largest one was used to calculate the egg index. For further explanation, see "Materials and Methods".

Taxon	Mean length (mm)	Range (mm)	Sample size	Mean max. diameter (mm)	Range (mm)	Sample size
Radoszkowskiana rufiventris	1.89	1.68–2.13	10	0.57	0.50–0.68	8
Coelioxys decipiens	2.09	1.90–2.30	6	0.75	0.65–0.90	6
Coelioxys coturnix	1.47	1.34–1.53	4	0.46	0.43–0.48	4
Megachile nigripes	4.35[a]	4.20–4.50	5	1.38[b]	1.20–1.50	4

[a]Based on four eggs and one mature oocyte.
[b]Based on three eggs and one mature oocyte.

decipiens have dimensions that overlap in both length and maximum diameter, so that to distinguish between them on size may be unreliable in many cases. As revealed in the descriptions, shape of oocytes may be variable in some cases, perhaps because of constraints imposed by follicular tissue or by dissection from this tissue.

Although somewhat thinner than that of *Coelioxys decipiens*, the egg/mature oocytes of *Coelioxys coturnix* presumably can be distinguished easily on the basis of size, not surprising since the adult body sizes of the two species studied here were quite dissimilar (intertegular distances of *C. decipiens* were 3.10–3.75 mm; those of *C. coturnix* were 2.25–2.70 mm).

Table 2 provides data regarding the egg indices, number of mature oocytes, and number of ovarioles per ovary of the four species treated here (*Radoszkowskiana rufiventris*, *Coelioxys decipiens*, their host *Megachile nigripes*, and *Coelioxys coturnix*). Data regarding *M. nigripes* require explanation. Of 11 adult females dissected, only 1 contained a mature oocyte even though many had frayed wings. To assemble the statistics for this species we measured the length of four eggs and the single mature oocyte and calculated an averaged length of 4.35 mm (table 1). We then divided this sum by the average intertegular distance of five adult females recorded in our notes to calculate an egg index of 1.02. (This value is close to the 0.99 egg index based on the length of the single mature oocyte divided by the intertegular distance of the female from which it was taken.) All females of this species had an ovariole formula of 3:3. The very low values of the average number of mature oocytes (column 3, table 2) and the average number of mature oocytes per ovariole (column 4, table 2) of this species are

TABLE 2
Egg Indices, Number of Ovarioles, and Number of Mature Oocytes/Eggs of Bees in Current Study

Taxon	Egg index	Size category	No. mature oocytes	No. mature oocytes per ovariole	Ovariole formula	No. specimens
Radoszkowskiana rufiventris	0.55	Small	2.71	0.45	3:3	7
Coelioxys decipiens	0.60	Small	3.00	0.50	3:3	6
Coelioxys coturnix	0.59	Small	2.75	0.48	3:3, 3:2[a]	4
Megachile nigripes[b]	1.02[b]	Large	0.09	0.015	3:3	11

[a]One of the four specimens of C. coturnix had the atypical ovariole formula of 3:2, but all others had the plesiomorphic formula of the family: 3:3. Yet another specimen of the same species, not included in the table, was completely sterile, with no tissue present within any of the ovarioles.
[b]Based upon the average length of one mature oocyte and four eggs taken from cells divided by the average intertegular distance of five adult females. For further information regarding data of this species, see "Discussion of Egg Anatomy and Ovarian and Egg Statistics".

because 10 females had no mature oocytes. We are uncertain why so many females of *M. nigripes* lacked mature oocytes. This phenomenon might relate to a slow pace of cell construction and provisioning; to the deposition of eggs at a certain time in the evening or night of the previous day, so that when females were netted at the nest site during the following day, their mature oocytes had already been deposited; or to some combination of these factors.

The egg indices of the three cleptoparasitic species are close, all categorized as "small", according to the classification of Iwata and Sakagami (1966). Information regarding the number of mature oocytes should not be given a great deal of attention beyond noting that, as is generally the case with parasitic bees, the number of mature or nearly mature oocytes in a female tends to be much greater than with solitary bees (see discussion in Rozen, 2003, and references therein). Thus, it is not surprising that the egg indices of these parasites are considerably smaller than that of the host, *Megachile nigripes*, which at 1.02 is categorized as "large". Among the cleptoparasitic specimens examined for this study, there were other oocytes that could be categorized as mature because the nurse cells had been absorbed, but the oocytes themselves were not counted because they were relatively small compared with other oocytes in the same individual.

To date, no cases have been discovered in the Megachilidae where the ovarioles have increased in number; all specimens had the basic formula of three ovarioles per ovary (with one exception, as noted above for *Coelioxys coturnix*). Increase in ovariole number beyond four (the basic number for all Apidae) is especially characteristic of the Nomadinae (Apidae) although it occurs infrequently in the Ericrocidini (Apidae: Apinae) (Rozen, 2003).

EVOLUTION OF CLEPTOPARASITISM IN THE MEGACHILINI

In addition to attempting to expand our knowledge of the biology and immature stages of *Radoszkowskiana*, we wanted to determine if such information might illuminate whether that genus had a common cleptoparasitic ancestor with *Coelioxys* or, conversely, whether cleptoparasitism evolved separately in the two genera. Females of *Radoszkowskiana*, because of their tapering metasomas, bear a strong resemblance to females of *Coelioxys*, and the two genera obviously are related, as indicated by shared adult characters (Michener, 2000). Much of the information regarding *Coelioxys* comes from a study by Baker (1971) dealing with *C.* (*Boreocoelioxys*) *octodentata* Say and *C.* (*B.*) *sayi* Robertson and from our investigation of *Coelioxys* (*Liothyrapis*) *decipiens* reported both here and elsewhere (Rozen and Kamel, 2006).

In summary, evidence presented here indicates that female *Radoszkowskiana rufiventris* enters the host nest when the host is away presumably gathering closure material, deposits her egg on top of the host egg, which is resting on the surface of the provisions, and then departs. Its embryo develops rapidly so that it hatches before the host does. Still surrounded by most of its chorion, it kills the embryonic host by biting it with strongly curved but short, fanglike mandibles that bear a tiny spined second tooth basally. Without moving its head, it then proceeds to ingest the entire contents of the chorion over a period of more than a day while remaining motionless on top of the host egg. Its body slowly swells as the host egg is depleted. Except for the fanglike mandibles and short incubation period, there are no other obvious adaptations of the first instar for its parasitic role. The second instar starts feeding on the provisions by moving the anterior part of its body to one side or the other of the deflated host egg. Its mandibles are apically bifid as are those of all subsequent instars, presumably adapted for feeding on the provisions.

In contrast, the works of Baker (1971) and Rozen and Kamel (2006) present the following biological synopsis of the species of *Coelioxys* that they studied. Their observations did not record when the cleptoparasite female enters the nest, but Michener (1953) observed *C. octodentata* attacking open host cells as did Graenicher (1927) in the case of *C.* (*Coelioxys*) *sodalis* Cresson. The *Coelioxys* egg is not in contact with the host egg and is either inserted into the leaf-lined cell wall or placed in or on

the provisions. The *Coelioxys* first instar is weakly sclerotized (and hence its cast exoskeleton is difficult to identify) and mostly pharate within the egg chorion. The second instar possesses somewhat enlarged mandibles but presumably does not ordinarily attack the host immature. Of all instars, the third has the most extensively developed mandibles bearing only a single apical tooth and other modifications of its head and is the one that aggressively and apparently often repeatedly attacks the host, which by that time presumably has hatched and started feeding (Michener, 1953; Bohart and Yousseff, 1972; Rozen, and Kamel, 2006). Fourth and fifth instars display the anatomy of a typical megachilid larva, with apically bidentate mandibles, and consume the provisions.

On the basis of this information, the incongruity between the modes of cleptoparasitism of *Radoszkowskiana* and *Coelioxys* would suggest that there is little evidence to assume that they had a common cleptoparasitic ancestor. The incongruities include: different host life stage attacked; different cleptoparasitic instar attacking; different behavior of attacking instar; different mandibular modifications of attacking instar.

However, Ferton's (1896) account of the mode of cleptoparasitism of *Coelioxys* (*Allocoelioxys*) *afra* Lepeletier shows a striking similarity with that of *Radoszkowskiana*. He discovered two instances where this cleptoparasite's egg was deposited with the anterior end on the host egg and the other end on the surface of the provisions when the host female had departed to gather leaf snippets to construct the next cell (or to close the cell). In one of these cases, he noted that two days later the eggs were in the same position, but in the evening of the second day he observed that the parasite's head had become defined and concluded that the egg had hatched. It then sucked the contents of the host egg, which subsided into the food, and by two days later was entirely empty. Afterward the parasite larva fed on the provisions.

From his account, we cannot be certain which instar attacked the host egg, and, for that matter, whether the host was still an egg, because of the confusion surrounding eclosion and young instars with so many early observations on these bees. Information on the anatomy of the larva and its mandibles is lacking. However, his description of how the host egg was depleted is highly suggestive of what we observed with *Radoszkowskiana rufiventris*, as is the timing of the events that he reported. Clearly a study of the biology and developmental anatomy of *Coelioxys afra* or some other species of *Allocoelioxys* is needed in order to explore the relationships of *Allocoelioxys* both with *Radoszkowskiana* and with other subgenera of *Coelioxys*. If it were discovered that the first instar of *C. afra* is the hospicidal form that attacks the host egg, then that mode of cleptoparasitism would seem to be well within repertoire of parasitism of *Coelioxys* and that this cleptoparasitic repertoire is indeed broad. On the other hand, if it were discovered that the third instar of *C. afra* attacked the host, which had already developed into a feeding instar, then there would be reason to think that *Radoszkowskiana* and *Coelioxys* both evolved from a nonparasitic ancestor, as suggested by Popov (1955) presumably on the basis of adult anatomy.

We have not considered Friese's (1923) observation on the egg of *Coelioxys rufescens* Lepeletier hanging by one end from the top of the cell of *Anthophora fulvitarsis* Brullé. We think that he probably mistook the egg of a *Melecta* (perhaps *M. armata* Pèrez since both he and Bischoff [1927] considered it a known cleptoparasite of *A. fulvitarsis*) for that of this *Coelioxys*. All known Melectini deposit their eggs in this way (see Rozen and Özbek, 2005, and references therein).

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